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SYMPATHETIC DENERVATION OF THE FEET AND LEGS OCCURRING SPONTANEOUSLY OR AS A RESULT OF DISEASE. A PRELIMINARY REPORT

HARRIS B. SHUMACKER, JR., M.D.

From the Department of Surgery, The Johns Hopkins University Medical School

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Although functional abnormalities of the sympathetic nervous system have been the subject of intensive study, little attention has been directed to anatomical lesions of the sympathetic nerves. The object of this communication is to make a preliminary report of five patients who have lost completely, either spontaneously or as a result of disease, the sympathetic innervation to various portions of the feet and legs. All the patients had peripheral arterial disease.

CASE REPORTS

Case 1. T. G. D. This white male, 59 years of age, was admitted to the hospital on December 2, 1940, complaining of diabetes, rectal incontinence, ulcers of the feet, and poor vision.

The family history revealed "uremic poisoning" and a cerebral vascular accident in the father, who died at 58, rheumatic fever in one sister, and hypertension in another sister. The patient had had influenza and pneumonia in 1909. In 1915 he began to have severe headaches, was found to have a positive blood Wassermann reaction, and was treated for three years, presumably with iodides and mercury. The headaches recurred from time to time. In 1921 the left side of the face suddenly became paralysed and dysphagia and dysphonia developed. The blood Wassermann reaction was positive. He was treated with neoarsphenamine with prompt subsidence of most of the symptoms. In 1925 he was again treated with neoarsphenamine because of ulcers in the throat. At this time there remained slight residual dysphagia, and weakness and numbness of the left side of the face. He had worn glasses for 13 years, and recently his vision had become blurred. In 1935 some urinary symptoms and weight loss developed and he was told he had diabetes. He was treated by dietary measures and insulin. In the fall of 1939 he noticed that his stools were sometimes hard and sometimes watery. A year later fecal incontinence, worse at night, began. There was some warning before defecation and some sphincter control, but he was not awakened by passage of a stool during sleep.

Five months before admission he had noticed an ulcer beneath the head of the first metatarsal bone on the right side and for six or seven weeks there had been an

ulcer over the left internal malleolus. He stated that he had no pain when resting and no intermittent claudication. For 30 years he had had calluses on the soles of his feet. He said that his feet formerly perspired freely but that he could not recall any perspiration at all in the feet for about 20 years. For six months there had been a little "numbness" of both feet, though this fact could be elicited only by direct questioning. He thought his feet had been a little cooler than usual during the past three or four months.

Examination: There were bilateral cataracts and early diabetic retinitis with small hemorrhages and evidence of early arteriosclerosis. The pupils were not irregular and reacted normally. There was moderate emphysema. The heart was not enlarged, the sounds were of fair quality, and there was a harsh systolic murmur loudest at the apex. The blood pressure was 118/60. There was a widespread area of hypalgesia of the body including the ulnar surfaces of the forearms,

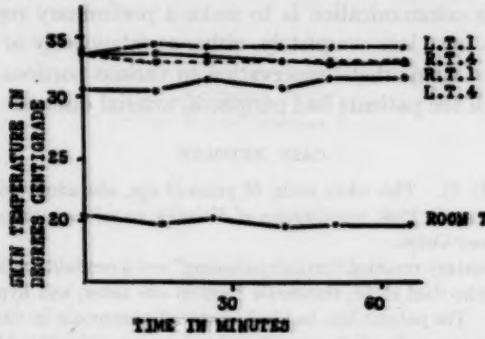

FIG. 1. SHOWING STABILITY OF TEMPERATURE OF TOES ON EXPOSURE TO COLD. CASE 1

the thorax below the fourth dorsal level, and the legs. Sensation to pain was diminished on the soles of the feet and he did not appreciate light touch on the feet or legs. Vibratory sense was impaired in the legs. There was no pain on compression of the tendo Achillis or testis. There was no ataxia. Ankle jerks, knee jerks, and biceps and abdominal reflexes could not be elicited. There was slight residual facial weakness on the left side.

The feet were warm and dry. There were some calluses, especially under the heads of the first metatarsals; in other places the skin appeared somewhat atrophic. A dirty perforating ulcer was present under the head of the first metatarsal on the right, and a similar ulcer over the left medial malleolus. The right dorsal pedal and left posterior tibial pulsations could be felt though they were diminished in volume. The left dorsal pedal and right posterior tibial pulsations were absent.

Skin temperature studies showed that the temperature of the toes was remark-

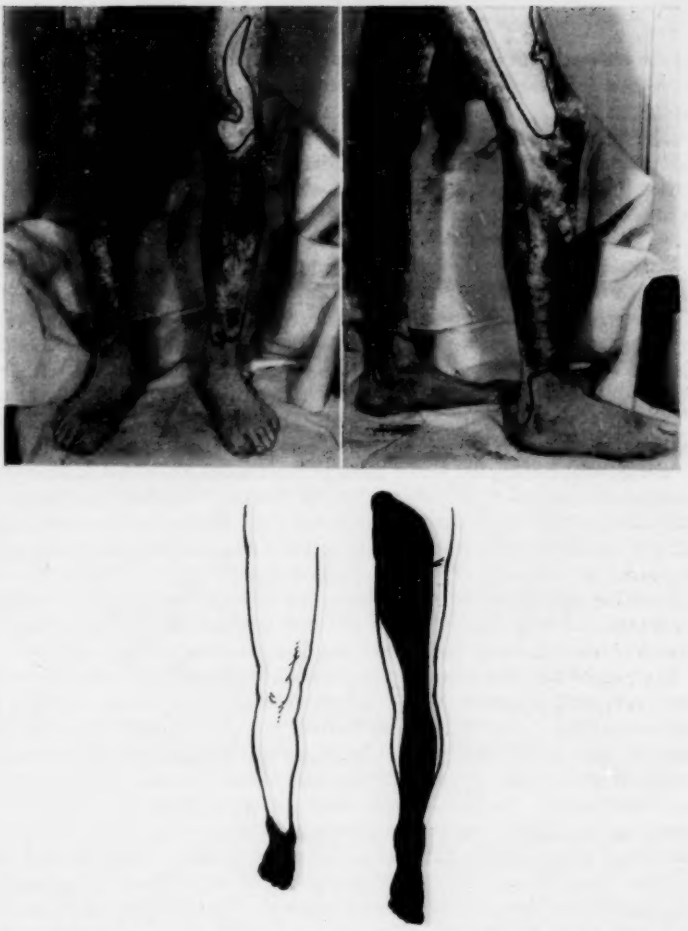

FIG. 2. SHOWING AREAS OF HIGH SKIN RESISTANCE AND OF ABSENCE OF SWEATING. CASE 1

The areas which sweat on exposure in a heating cabinet are dark (starch-iodine). The areas distal to the pencil mark on the legs and within the areas outlined on the thigh are areas in which cutaneous resistance to two microamperes was too high to measure (greater than 3,800,000 ohms). The close association of high resistance and absence of sweating is apparent. For comparison, pen and ink sketches of the area of absent sweating and high skin resistance after 5th lumbar ganglionectomy are included (5).

ably stable on exposure to a cool room temperature (Fig. 1). The temperature of the toes varied from 31 to 34°C. and on exposure to a room temperature of 20°C. for over an hour the temperature of the toes remained essentially the same; the temperature of several toes actually increased a fraction of a degree and in no instance did it decrease more than 0.6°C. Skin resistance studies and sweating tests demonstrated that there was absence of sympathetic innervation to both feet and also to an area on the lateral surface of the right thigh and the anterior surface of the left thigh. (Fig. 2.)

Hematological studies were essentially normal. The blood Wassermann reaction was negative; an Eagle flocculation and Kline tests were doubtful on one occasion, negative on another; and the spinal fluid Wassermann was negative. Blood chemistry and urine examinations were not remarkable except for evidence of diabetes. Roentgenograms of the heart, aorta, and lungs were negative. There was no calcification of the vessels of the feet but there was calcification of the arteries of the legs. There was some osteitis of the left tibia, thought to be due to syphilis.

The diabetes was regulated; the cataracts were removed; the incontinence was corrected by enemata given twice daily. With warm compresses to the ulcers, salicylates to the calluses, lanolin daily to both feet, Buerger-Allen exercises, and bed rest the ulcers healed nicely and the calluses disappeared. The patient was discharged on February 8, 1941, much improved.

It was felt that the patient had diabetes mellitus, cataracts, mild diabetic retinitis, general and peripheral arteriosclerosis, and ulcers of the feet; and it was the opinion of some observers that he had syphilitic osteitis and tabes dorsalis.

The patient was readmitted on June 2, 1941. Apparently he had been somewhat negligent in regard to both his feet and the diabetes. For two months there had been vesicles on the left heel, the plantar surface of the right foot, and the first, third, fourth, and fifth right toes. The lesions on the right foot had become infected and gangrenous. On examination there was seen an ulcer on the left heel and black, infected, gangrenous areas were present over most of the first, third, fourth, and fifth right toes and over a large area on the sole of the right foot. There was cellulitis extending to the ankle. The urine contained sugar (3 plus) and acetone. A supracondylar amputation was carried out under spinal anesthesia. This was followed by a satisfactory convalescence. He was discharged on June 21, the diabetes having been regulated and the ulcer on the left heel having improved considerably. A month later the ulcer was healed.

Case 2. J. C. S. This white male, 45 years of age, was admitted to the hospital on December 12, 1940, complaining of diabetes, recurrent thrombophlebitis of the left lower extremity, and ulcers on the left foot.

For a year and a half the patient had known that he had diabetes. Its course had been mild.

For 20 years he had been troubled by the arches of his feet and had had a great deal of discomfort in the region of the metatarsal arches. He had worn various

sorts of arch supports. For five or six years there had been calluses on both feet, especially under the heads of the first metatarsals and on the great toes with open sores in these calloused areas for a year and a half. At times there was a very bad odor. During the past three months he had been in bed a great deal and the ulcers on the right foot had healed. He stated that the ulcers sometimes formed in this manner: a blister would appear which would turn black, become dry, and then ulcerate. There was no pain when resting and no intermittent claudication, only an aching pain in the region of the ulcers on walking. Interestingly enough, he said that his feet had formerly perspired so profusely that he often had to change shoes as well as socks during the day. For the past five years, however, there had been no sweating of the feet. Upon direct questioning he stated that in recent years he thought he had less feeling in the soles of his feet than formerly. He had noticed nothing unusual about the temperature of his feet.

During the past several years he had noticed large dilated veins in the left leg. A little discomfort associated with them was relieved by an elastic stocking which he had worn for three months. He had no sense of fatigue and no pruritis. There had been some swelling of the ankle which was largely relieved by the use of an elastic stocking. There had been no treatment for the veins.

In the fall of 1940 he had had three attacks of left femoro-iliac thrombophlebitis, each lasting about two weeks. The last occurred after extraction of teeth. He was given sulfanilamide during each attack.

Examination: The patient was a large man, somewhat overweight. The general examination was not remarkable. The femoral and popliteal arteries were pulsating well and pulsation could be felt in both posterior tibials. There was no pulsation in either dorsal pedal artery. The feet were dry and surprisingly warm; there was no edema. The nails were moderately thickened. There was a thick callus under the center of each metatarsal arch. There was a deep crater in the middle of the one on the left foot running longitudinally, and near the distal end the ulcer was especially deep and dirty. Calluses were present along the mesial border of both great toes and there was a healing ulcer on the left. The longitudinal arches were adequate, but there was depression of the metatarsal arches and some pronation of both feet. There were moderate varices in the left leg and the saphenous vein was dilated as high up as the thigh. The system filled readily when the extremity was dependent and emptied promptly when it was elevated, and a tourniquet about the upper thigh prevented this filling. Neurological examination was essentially negative except for absence of sensation to light touch and pin prick in the soles of the feet. Vibratory sensation seemed somewhat reduced over the patellae and malleoli. Tendon reflexes were somewhat sluggish but were present and equal on the two sides.

Skin temperature studies showed a remarkable stability of the temperature of the toes on exposure to cold. There was no response to body heating (Fig. 3). Skin resistance and sweating tests revealed absence of sweating and very high cutaneous resistance over almost the entire extent of both legs and feet (Fig. 4).

Hematological studies were essentially normal; the blood Wassermann reaction

was negative; the urine examination and blood chemistry studies were negative except for the characteristic changes of diabetes; phenolsulfonphthalein excretion was 80 per cent in two hours; and roentgenograms of the chest revealed normal heart, aorta, and lungs. No calcification of the vessels of the forearm was seen in the roentgenograms, but some calcification of the arteries in the legs was visible. An electrocardiogram was essentially normal. Culture of the ulcers of the feet yielded B. proteus, and that of the tonsils beta hemolytic streptococci and H. influenzae. A diagnosis of hyperopia with astigmatism and presbyopia and of mild diabetic retinitis was made by an ophthalmological consultant. An oto-laryngological consultant thought the tonsils and adenoids were chronically infected. Several diseased teeth were found.

The diabetes was regulated and the patient was put upon a reducing diet. On December 26 a dental extraction was performed. Immediately after this there was a recurrence of the phlebitis together with a rise in temperature to 103°F.

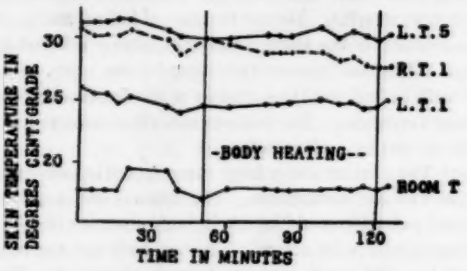

FIG. 3. SHOWING THE BEHAVIOR OF SKIN TEMPERATURE OF THE TOES ON EXPOSURE TO COLD AND THE LACK OF EFFECT OF PROLONGED BODY HEATING. CASE 2

The attack subsided in about three days. On January 21 tonsillectomy and adenoidectomy was performed. Before this operation the patient had been given sulfathiazole. There was no phlebitis. A week later the left saphenous vein was ligated and a retrograde injection of sodium morrhuate was given which effected good obliteration of the varicosities. At operation much scarring was seen about the femoral vein as a result of the recent thrombophlebitis. With saline soaks, surgical paring of the calluses, and application of salicylates the ulcers healed completely and the calluses disappeared. The patient was provided with metatarsal bars and was discharged on February 6. He was urged without success to give up smoking.

He returned for study in January 1942. The callus on the side of the left foot had recurred and a fissure had developed in it six weeks after discharge. In April he had sustained a fracture of the distal ends of the first three metatarsals and of the phalanges of the great toe of the left foot. This was followed by osteomyelitis.

FIG. 4. SHOWING AREA OF HIGH CUTANEOUS RESISTANCE AND OF ABSENCE OF SWEATING IN CASE 2

These areas are represented in pen and ink sketches in A and B. For comparison, the areas of high resistance and of absent sweating following third and fourth lumbar ganglionectomy are shown in sketches C and D. The resemblance of the areas of sympathetic denervation on the anterior surface of the patient's leg to those which result from operation is apparent. In this patient the posterior surface of the thigh, which is always denervated by lumbar ganglionectomy and which represents the second and third sacral segments, had normal sympathetic innervation.

He had recovered from this as the result of incision and drainage, and sulfonamide treatment. In the summer of 1941 he had had two brief mild attacks of left femoral phlebitis. On examination the feet were warm and dry and the

posterior tibial pulsations were still present. Skin resistance studies showed the areas of high resistance to be essentially the same as on examination a year previously.

Case 3. S. S. This Russian Jewish male, 49 years of age, came to the surgical dispensary in January, 1941, complaining of cramps in the right leg on walking. He had been quite well until 1927 when motor aphasia and right hemiparesis developed suddenly. He was said to have had transient hypertension during the first week of this illness. Recovery was rapid, but seven weeks later another severe accident occurred with complete motor aphasia and paralysis of the right side resulting. The blood pressure was normal before the first stroke and during and after the second. He recovered slowly and seven weeks later had no difficulty with speech but he continued to have slight stiffness and weakness of the right

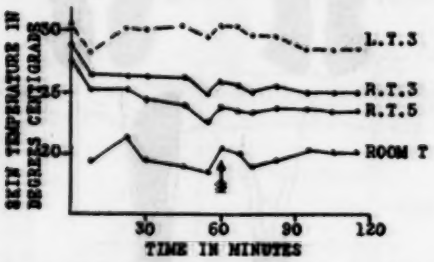

FIG. 5. SKIN TEMPERATURE STUDIES IN CASE 3

The temperature of the feet was relatively stable on long exposure to cold, and an effective posterior tibial block brought about no alteration in skin temperature. The arrow indicates the time of the procaine injection.

leg and minimal changes in the right arm. He had had no headaches, sphincter difficulty, or trouble with speech. A year after the second stroke he had been given some injections in the buttocks which he said were intended to relieve the residual weakness. During the course of treatment he began to have epigastric pain, worse during periods of emotional stress. There were no bloody or tarry stools. Because of this abdominal pain he had been admitted to the medical service in October 1940.

At that time examination showed slightly increased resistance in the right arm without pyramidal signs, slightly increased resistance in the right leg, and absent associated movements of the right toes. The Babinski sign was absent. There was vague diminution in two-point discrimination in the hand and slight diminution in perception of pin prick and light touch, and there was similar hypalgesia of the feet. Otherwise examination was not remarkable. The Wasserman reaction was negative; roentgenograms of the chest were negative; gall bladder

visualization showed normal function, and no stones; the gastro-intestinal series revealed a penetrating lesion of the lesser curvature thought to represent a pyloric ulcer. Stool examinations were negative for blood. Roentgenograms of the head

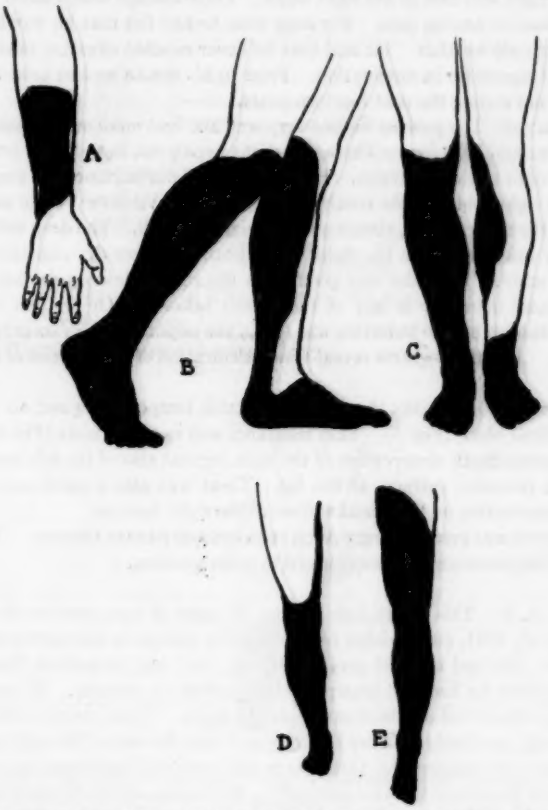

FIG. 6. SHOWING THE AREAS OF HIGH CUTANEOUS RESISTANCE AND OF ABSENT SWEATING IN CASE 3

For comparison with the area in the right leg, a sketch of the extent of sympathetic denervation which is brought about by second and third lumbar ganglionectomy is shown in D and E.

and sella were normal. The basal metabolic rate was −2; the non-protein nitrogen was 37 mgm. per cent. He was discharged much improved. The diagnosis was peptic ulcer, residual right hemiparesis, and psychoneurosis.

Some time after discharge he began to feel cramps in the right calf on walking. At the time he was seen he stated that he could not walk more than one block without having severe cramps in the calf. Sometimes the same cramps were also felt in the right foot and in the right thigh. These cramps disappeared with rest, and there was no resting pain. For some time he had felt that his hands and feet were cool in cool weather. He said that he never recalled sweating in either foot. He smoked cigarettes in moderation. Prior to his stroke he had used alcohol to excess but not during the past fourteen years.

Examination: The patient walked very well and had moderately good strength in both arms and both legs. There was slight hypalgesia in the right arm and leg and in the right half of the trunk. He could not feel pin prick or light touch on the sole of the right foot or light touch on the dorsum. Vibratory sense was diminished over the right patella, absent over the right malleoli. The deep reflexes were moderately exaggerated on the right side. Both feet were dry and the skin was somewhat scaly. The color was good. On the right side no pulsation was felt in the femoral artery or in any of the vessels below it. On the left there was femoral pulsation but no pulsation was felt in the popliteal artery or in the vessels of the foot. Roentgenograms revealed no calcification of the arteries of right foot or leg.

Skin temperature studies showed rather stable temperatures and no rise after posterior tibial block (Fig. 5). Skin resistance and sweating tests (Fig. 6) showed complete sympathetic denervation of the right leg and also of the left foot and the lateral and posterior surfaces of the leg. There was also a small area of sympathetic denervation on the dorsal surface of the right forearm.

The patient was given Buerger-Allen exercises and pavaex therapy. There was only slight improvement. He was urged to cease smoking.

Case 4. A. S. This Polish iron worker, 51 years of age, came to the surgical clinic in April, 1941, complaining of intermittent cramps in the calves and a sore on one toe. He had enjoyed good health and had had no serious illness. For about two years he had had cramps in both calves on exercise. He could walk about three blocks before claudication would begin. There was no resting pain. About a week previously an ulcer had appeared near the nail of the right great toe. His feet had not perspired for three years although they had done so previously. His legs have continued to sweat normally. He smokes about 25 cigarettes a day.

Examination: The general examination was not remarkable. The feet were dry. The right foot was cooler than the left but both were moderately warm. There was moderate dependent rubor of the right foot. Neither dorsal pedal nor posterior tibial pulsation was felt. On the mesial side of the nail of the great toe there was a small dry ulcer. Roentgenograms revealed no calcification of the arteries of the leg or foot.

Skin temperature studies showed the temperature of the feet to be remarkably stable and uninfluenced by posterior tibial nerve block (Fig. 7). Skin resistance and sweating tests showed complete sympathetic denervation of the sole of the

right foot except for the proximal portion of the heel and of most of the sole of the left foot. There was also almost complete sympathetic denervation of the distal portion of the left sole and of the left dorsum as evidenced by increased skin re-

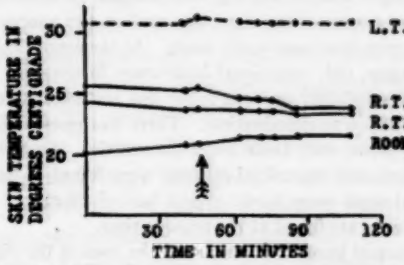

FIG. 7. SKIN TEMPERATURE STUDIES IN CASE 4

The temperature changed little with exposure to cold and with posterior tibial block. The arrow indicates the time of procaine injection.

FIG. 8. SHOWING AREAS OF HIGH SKIN RESISTANCE AND OF ABSENT SWEATING IN CASE 4

The skin resistance was too high to read and no sweating occurred in the shaded areas. In the cross-hatched areas there was only a faint trace of sweating and skin resistance was very high (nearly 3,800,000 ohms).

sistance and diminished sweating (Fig. 8). It was thought that the patient had Buerger's disease. He was given pavaex treatment with little benefit. The tiny ulcer healed and recently has recurred and has become rather painful. There is no osteomyelitis. At present the ulcer appears to be healing.

Case 5. R. C. This white male, 77 years of age, was known to have had diabetes for 17 years. In 1930 one observer had found signs which he thought were suggestive of diabetic neuritis, a positive Romberg, difficulty in sharp-dull differentiation on the back, right arm and left leg, some diminution in perception of cotton-wool on the posterior aspect of the right arm and on the antero-lateral aspect of the left leg, and sluggish knee and ankle jerks. At the same time other observers found less definite signs, only occasional inaccuracy in response to pin prick and confusion of thermal sensibility on both lower legs and back, findings which were not definitely delimited on re-examination. There was questionable, slight diminution in vibratory sense over both lower extremities. On re-admission in December 1940 the deep and superficial reflexes were found to be normal and no positive neurological signs were found except loss of vibratory sense below the knees. Only these signs are found at the present time.

The patient developed gangrene of some of the toes of the right foot and underwent a supracondylar amputation in January 1941. Before this was carried out skin temperature studies were done. Because of the history of absence of sweating in the feet for several years and unusual stability of the temperature of the toes on exposure to cold, it was suspected that this patient might have undergone loss of sympathetic innervation of the feet. Roentgenograms of the legs had shown extreme calcification of the arteries.

In February, 1942 there was little change in the left foot except that the circulation was a little poorer than on the former examination. He had been complaining of some pain. Skin resistance studies showed extremely high resistance of the left foot and the lower half of the leg with the exception of a small area of lowered resistance about 4 cm. in diameter over the external malleolus.

I have seen one additional patient in whom I believe a similar defect of sympathetic innervation of the feet and legs exists. I have not, however, studied this patient in sufficient detail to permit me to be certain of the exact extent of sympathetic loss.

DISCUSSION

The cases presented have no common factor except obliterative arterial disease which might be construed to be of etiological significance in regard to the loss of sympathetic innervation. Three patients were diabetics, and one of these had late syphilis. One patient had slight residual signs of an old hemiplegia; a second patient had had, in addition to diabetes and syphilis, an old cerebral vascular accident associated only with a transient facial palsy, dysphagia, and dysphonia. None of the patients had complaints suggesting peripheral neuritis. Objectively, there was no loss of sensation in Case 4. In

Case 3 there was diminution in sensation on the hemiplegic side, but the loss of sympathetic innervation was bilateral and occurred on the contralateral side where no sensory impairment was demonstrable. In Case 2 there was hypalgesia on the soles of the feet but the sympathetic denervation included almost the entire legs, being just as complete in areas with perfectly normal sensation as on the soles of the feet. In Case 1 there was extensive hypalgesia but there was no correlation between the sensory loss and the sharply delimited areas of loss of sympathetic innervation. Two of the patients had thromboangiitis obliterans and three had peripheral arteriosclerotic disease.

Sympathetic innervation is lost in a certain distribution whenever the continuity of a peripheral nerve is completely interrupted by either trauma or disease. In such cases the area of sympathetic denervation corresponds to the area of innervation of the affected nerve. List and Peet (1, 2, 3) and Hyndman and Wolkin (4) have studied the defects in the sweating mechanism in cases of lesions of the brain stem, cord, sensory and sympathetic nerves. Wilkins and Kolb (5), stimulated by observations of abnormal vasomotor function in cases of beriberi, studied the vasomotor reactions in 15 cases of peripheral neuritis. In half of these patients there was some decrease in vasomotor tonus in the feet, comparable to what had been observed by these workers in incompletely sympathectomized extremities. Definite improvement in vasomotor activity occurred during two weeks of appropriate treatment, a result suggesting that a significant part of the abnormality was not due to anatomical alteration of the sympathetic nerves. Full recovery was, however, slow or failed to be achieved. There was no rigid relationship between the degree of vasomotor disturbance and loss of sensation or muscular power although in general the more severe cases were the ones with vasomotor abnormalities. Wilkins and Kolb observed no instance where sympathetic innervation was completely absent from a part.

It has been apparent since the initial report of Bradbury and Eggleston (6) that cases of postural hypotension are associated with alterations in the sympathetic nervous system. The absence of reflex postural vasoconstriction, the hypohydrosis or anhydrosis which is so commonly present, and the loss of reflex cardiac acceleration suggest that the disorder is due to some fundamental disturbance in the

sympathetic nervous system. Whether the disturbance is in the peripheral nervous system (6, 8, 9) or in the central pathways is not known (7, 10). Although most investigators have been interested largely in the general effects of the altered function of the sympathetic nerves there is evidence that in some patients with postural hypotension there may be complete sympathetic denervation to certain parts of the body. Bradbury and Eggleston stated that the anhydrosis observed in their patients was not due to a defect in the sweat glands or to lack of their ability to respond to pharmacological stimulation of the sympathetic endings. Others have also demonstrated areas which do not sweat upon exposure to heat but which sweat after administration of pilocarpine (7–9). Chew, Allen and Barker (9) had one patient in whom, on exposure to heat, sweating occurred only above the level of the umbilicus. In one of the patients reported by Stead and Ebert (10) there was no sweating below the ankles when the body was heated and very little sweating along the anterior surfaces of the thighs and legs. The skin temperature of the feet was remarkably stable on exposure in a cool room, and there was no increase or decrease in blood flow of the feet on heating or cooling the body as determined by plethysmographic study. The vasomotor and sudomotor responses in the upper extremities were normal. It is of interest that some of the patients with postural hypotension have tabetic or other lesions of the central nervous system. Among my cases there were no patients who had symptoms of postural hypotension. Blood pressure determinations with alteration in position of the patients were not carried out.

Samuels (11) has noted the sudden cessation of perspiration in the affected foot in peripheral circulatory deficiency. I have often seen this symptom in patients with obliterative arterial disease. As far as I know, this cessation is not indicative of sympathetic nerve loss to the affected part, such patients exhibiting nothing else to suggest such a mechanism. Whether it is due to local nutritive difficulties in the sweat glands I cannot say. As Samuels has pointed out, in such cases the feet often resume normal sweating as the peripheral circulation is improved.

It is impossible to say what is the nature of the lesion which produces the sympathetic denervation in the patients described, or what

is the site of the lesion. Perhaps, indeed, the nature and the site of the lesion were not the same in all five cases. Theoretically, the lesion might be located in the brain or cord, in the anterior roots, in the white rami communicantes, in the sympathetic ganglia, in the sympathetic chain, or in the sympathetic fibers running in the peripheral nerves. It is difficult to imagine a lesion of the cord which would single out for destruction only the sympathetic fibers and leave free of injury all the adjacent nerves. Similarly it is difficult to conceive of a lesion affecting numerous peripheral nerves in such a manner as to destroy completely the sympathetic supply to a part, with no injury at all to the sensory or motor fibers. It will be remembered that in 3 of the 9 extremities affected there was no demonstrable sensory or motor loss, and that in two other extremities hypalgesia was limited to the soles of the feet whereas the sympathetic denervation affected almost the whole of the legs. Without further information, one would feel that a more likely explanation is that the lesion is located in the sympathetic rami, ganglia, or chain.

As is indicated by the sketches of the area of sympathetic denervation occurring after excision of various sympathetic lumbar ganglia, there is a resemblance between such defects and those which occurred in the patients presented. In Case 3 the area denervated on the right leg resembled that seen after excision of the second and third ganglia; that present in Case 2 was not unlike the area seen after third and fourth ganglionectomy; and that present in Case 1 and also in the left leg in Case 3 bore some resemblance to the area of denervation following fifth lumbar ganglionectomy (12). In general, the chief difference lies in the freedom of the posterior surface of the thigh from involvement. This area, representing the second and third sacral segments, is always denervated when any portion of the lumbar sympathetic chain is interrupted by excision of a ganglion.

In my patients I have used criteria of sympathetic denervation which have been found valuable in the study of many patients who have been subjected to operative sympathectomy. The stability of skin temperature on exposure to cold and the inability to influence the skin temperature by such tests as body heating and peripheral nerve procaine block are suggestive. The absence of sweating with the Minor test and the occurrence of extremely high cutaneous resistance

under conditions which favor the lowest possible skin resistance (after heating the entire body in a heating cabinet until general sweating is profuse), we have found to be regularly present in areas which have been operatively denervated. Both absence of sweating and high cutaneous resistance under the conditions of the test have not been found to occur except when the sympathetic innervation has been interrupted by anesthesia (13) or by disease or operation.

How common the clinical picture described may be, only time and careful observation will tell. How it influences the course of the patient's health must also await further study. From the few cases observed, one would judge that it tends to influence the circulation favorably, as one would expect. If there is little enough occlusive disease of the peripheral arteries, or, to put it another way, if there are sufficient patent blood vessels in the vascular bed to permit an adequate circulation when they are kept in maximal vasodilatation, this naturally occurring sympathetic loss should help in the same manner as does sympathectomy in those cases of occlusive disease with a significant element of vasospasm. Indeed, the circulation in the feet of some of these patients appeared to be better than the state of calcification and the occlusion of major arteries would have led one to expect. Apparently there is no very great beneficial effect on intermittent claudication, just as is often true after operative sympathectomy. The patients have a tendency to the usual difficulties associated with absence of sweating, thickening of the skin, calluses, and so on, and must be urged to keep the skin greased with lanolin or some similar preparation.

SUMMARY

The case histories are presented of five patients in whom complete loss of sympathetic innervation to various portions of the feet and legs had occurred either spontaneously or as a result of disease.

The author wishes to express his thanks to Dr. C. P. Richter and to Mrs. B. G. Woodruff for their helpful cooperation in carrying out the skin resistance and Minor tests.

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ULTRACENTRIFUGATION OF THE ANTIDIURETIC, CHLORURETIC, AND PRESSOR FACTORS OF POSTERIOR PITUITARY EXTRACTS[1]

GEORGE C. HAM[2] AND MORRIS ROSENFELD

From the Department of Internal Medicine, University of Virginia and the Department of Pharmacology and Experimental Therapeutics, The Johns Hopkins School of Medicine

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Van Dyke, Chow, Greep and Rothen (1, 2) have recently succeeded in isolating a pure protein with constant oxytocic, pressor and diuresis-inhibiting activities from the pars neuralis of the ox pituitary. Evidence had been presented by Rosenfeld (3) that the pressor and oxytocic factors in untreated press juice of the posterior lobe of the pituitary gland could be readily concentrated by ultracentrifugation. On the other hand, these same factors in chemically treated extracts such as boiled press juice, pitruitrin, pitressin and pitocin were only very slightly concentrated under the same conditions.

In an independent study, Ham and Landis (4) found that the antidiuretic and chloruretic factors in pituitrin were not appreciably concentrated by ultracentrifugation. The present study was undertaken to compare the sedimentation properties of the antidiuretic, chloruretic and pressor factors in untreated press juice and in chemically treated extracts of the posterior lobe.

METHODS

Press juice was prepared from fresh posterior lobes of beef pituitary glands in a manner previously described (3). The press juice was made up with 0.9 per cent solution of sodium chloride to represent a 10 per cent extract of fresh gland (pH about 7.0). Another form of untreated extract was prepared by grinding fresh gland with a little sand in ten times its weight of 0.9 per cent sodium chloride solution.

A portion of the press juice was acidified with glacial acetic acid to a concentration of 0.25 per cent (pH 3.5–4.0). The solution was placed in a bath of boiling water for 30 minutes and then was neutralized to pH 7.0 with sodium hydroxide.

Pitressin (Parke, Davis and Co.) represented a commercial chemically purified product.

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[2] Commonwealth Fund Fellow, 1940–41.

Samples of each of these preparations were ultracentrifuged in a Beams air-driven ultracentrifuge at 60,000 R.P.M. for a period of seven hours. The solutions in runs I, II, and III (Table 1) were separated into an upper fraction of 3 cc. and a lower fraction of 1.6 cc. The solutions in run IV were divided into three equal fractions in order to demonstrate more sharply the difference in concentration between the top and bottom fractions. Fractionation was accomplished by means of a special partitioned type of ultracentrifuge cell (5).

To assay antidiuretic activity the rat method of Burn was used with modifications to increase its accuracy and dependability. The procedure has been described in detail by Ham and Landis (4). In each experiment 18 male white rats which had been fasted for twelve hours were used. Six animals were used to

TABLE I

MATERIAL	RUN	A.D. ACTIVITY (SQ. INCHES)		CHLORURETIC ACTIVITY				PRESSOR ACTIVITY (CONC. RATIO)	
				Eq. chlorides		Conc. ratio			
		Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
Press juice	I	1.2	4.8	79	230	1	9	1	5
Press juice	II	4.9	10.1	67	218	1	12	1	6
Press juice	III	2.2	7.9	154	294	1	10	1	8
Press juice	IV	.6	5.5	37	161	1	11+	1	25+
Saline extract	I	1.2	8.2	26	191	1	6+		
Boiled P.J.	I	8.2	8.0	177	174	1	1.5	1	1.5
Boiled P.J.	II	5.8	5.3	78	125	1	2	1	2
Boiled P.J.	III	6.5	7.9	98	148	1	2	1	2
Boiled P.J.	IV	7.0	7.5	58	126	1		1	2
Pitressin	I	11.6	11.3	120	136	1	1	1	1.5

Eq. = microequivalents per 100 gms. of rat during 3 hour collection period. Conc. ratio = concentration ratio. A.D. = anti-diuretic. P.J. = press juice.

assay each of the three solutions involved in an experiment: the top fraction, the bottom fraction, and an inactive control solution. The six rats were divided into two groups of three animals and each group was placed in a separate cage for the collection of urine. The animals were given a priming dose of water by gavage equivalent to 2½ per cent of their body weight two hours before the assay was performed. At zero time (see Fig. 1) a water load equivalent to 5 per cent of body weight was given by gavage and the solutions to be assayed were injected intraperitoneally. The injected solutions represented a concentration of fresh gland from 1–20,000 to 1–60,000. The cumulative volumes of urine expressed as cc. per 100 gms. of rat, excreted by the control and experimental groups were plotted at 15 minute intervals. The area enclosed by each curve was measured

by means of a planimeter. The difference between the control area and the experimental area represented antidiuretic activity.

The total chloride excretion during the three hour period by each group of three animals, expressed in microequivalents per 100 gms. of rat, was determined by the Van Slyke and Sendroy (6) modification of the open Carius method.

Pressor activity was standardized by means of the blood pressure response in cats anesthetized with sodium phenobarbital.

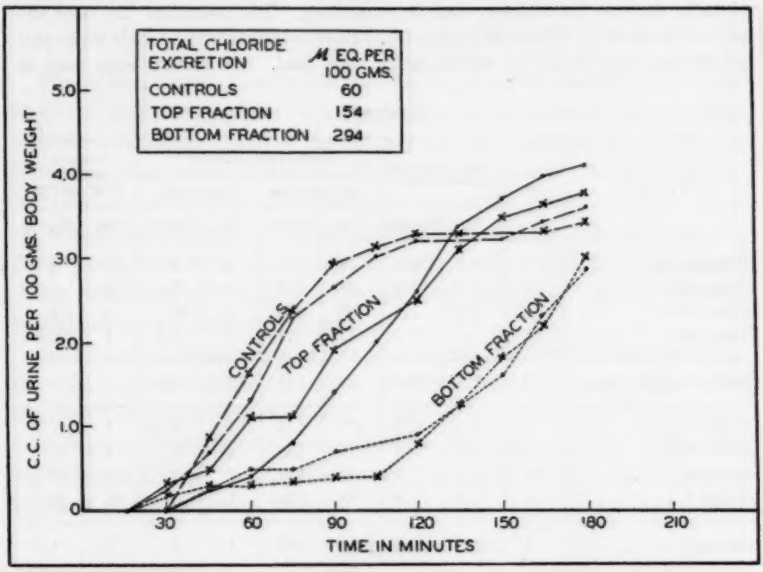

FIG. 1. ANTIDIURETIC AND CHLORURETIC TESTS ON ULTRACENTRIFUGED PRESS JUICE OF THE POSTERIOR PITUITARY GLAND (RUN III OF TABLE 1)

RESULTS

The chart (Fig. 1) shows the results of a typical experiment with ultracentrifuged press juice from run III. The average of the areas subtended by the two control groups measured 12.9 sq. inches. The average of the areas subtended by the curves of the two groups receiving the top fraction measured 10.7 sq. inches, representing a slight antidiuretic activity of 2.2 sq. inches (12.9–10.7). The average of the areas subtended by the curves of the two groups receiving the bottom fraction measured 5.0

sq. inches and represented a marked antidiuretic activity of 7.9 sq. inches (12.9–5.0).

The chloruretic activity of the top fraction is represented by a total chloride excretion of 154.0 microequivalents as compared with 294 microequivalents for the bottom fraction and 59.5 microequivalents for the controls. The total chloride excretion of controls in all experiments averaged 40 microequivalents and never exceeded 89 microequivalents per 100 gms. of rat. A dosage-response curve obtained with pituitrin permitted interpretation of the data in terms of approximate dosage ratios. It is evident from the curves that although both top and bottom fractions showed antidiuretic and chloruretic activity the bottom fraction was much more active.

In table 1 we have selected 10 representative experiments out of a total of 32.

The first four experiments in this table show the results on ultracentrifuged untreated press juice. In each case the antidiuretic, chloruretic and pressor activity of the bottom fraction was considerably greater than that of the top fraction. In the case of the chloruretic and pressor assays approximate concentration ratios could be assigned. Because of the wide limits of error of our assay methods the ratios obtained in the chloruretic and pressor assays are not regarded as significantly different. The antidiuretic assay indicated a marked concentration of active material in the bottom fraction but the data did not permit interpretation in terms of concentration ratios.

A similar concentration of active factors in the bottom fraction was obtained in the ultracentrifuged simple saline extract. Nine other experiments with four different saline extracts gave essentially the same results.

The sedimentation properties of the antidiuretic, chloruretic and pressor factors were definitely altered by boiling the press juice, acidified with 0.25 per cent acetic acid, for 30 minutes. In each of the four experiments on boiled press juice the bottom fractions show very little increase in antidiuretic activity as compared to the top fractions. The chloruretic and pressor assays indicate only a slight degree of sedimentation. The antidiuretic assay shows practically no sedimentation. The antidiuretic assay is less sensitive to small differences in dosage than the chloruretic and pressor tests.

Ultracentrifugation of pitressin gave results essentially the same as

those obtained with boiled press juice. The chloruretic and pressor activities of the bottom samples were slightly greater than those same activities in the top fractions, showing evidence of only slight sedimentation. The antidiuretic assay method again appeared to be less sensitive and gave no evidence of sedimentation.

DISCUSSION

The sedimentation properties of the antidiuretic and chloruretic factors in ultracentrifuged pituitrin are reported elsewhere by Ham and Landis (4). That study showed that ultracentrifugation at 60,000 R.P.M. for periods from 3 to 6 hours failed to produce a measurable degree of sedimentation. Rosenfeld (3) reported similar behavior on the part of the pressor and oxytocic factors in pituitrin.

The present study confirmed these findings in that chemically treated hormone as represented by boiled acidified press juice and pitressin showed only slight sedimentation of the antidiuretic, chloruretic and pressor factors.

In marked contrast, the antidiuretic, chloruretic and pressor factors showed a high degree of sedimentation in untreated press juice. Within the wide limits of the assay methods the sedimentation of all three factors appeared to be of the same grade.

It is evident that the three factors are present in untreated press juice in a form readily sedimented by ultracentrifugation at 60,000 R.P.M. for a period of 7 hours, whereas chemically treated extracts as represented by boiled acidified press juice and pitressin contain the antidiuretic, chloruretic and pressor factors in a relatively non-sedimentable form. These observations are in agreement with the findings of van Dyke, Chow, Greep and Rothen (1, 2) who have identified the activities with a protein of molecular weight 30,000.

SUMMARY AND CONCLUSIONS

The antidiuretic, chloruretic and pressor factors in untreated extracts of the posterior lobe of the pituitary gland can be concentrated by ultracentrifugation at 60,000 R.P.M. for 7 hours.

These three factors, when subjected to drastic chemical treatment, in extracts such as boiled press juice and pitressin, fail to show appreciable concentration by the same degree of ultracentrifugation.

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INVESTIGATION INTO THE MECHANISM OF THE WELTMANN SERUM COAGULATION REACTION

A Preliminary Report

SIDNEY SCHERLIS, M.D., and DEXTER S. LEVY, M.D.

From the Department of Medicine, The Johns Hopkins University School of Medicine

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There are a number of empirical non-specific tests which are used as aids in the diagnosis of diseases and in following their clinical courses, such as the formol-gel tests, the Takata-Ara reaction, the Hanger cephalin flocculation test, and the erythrocyte sedimentation rate. The Weltmann serum coagulation test is a non-specific test, which differs from these in that it is used in an attempt to differentiate between exudative and proliferative reactions.

Frequent Weltmann tests were carried out in a group of patients who had recently had rheumatic fever in an effort to discover possible subclinical rheumatic activity and to study more closely the development of valvular lesions. Six hundred separate results were recorded. These will be reported separately. Repeated use of this procedure stimulated investigation of its underlying mechanism. These experiments form the basis of this preliminary report.

The serum coagulation test was devised by Weltmann (1) in 1930. Other investigators had shown that the coagulation temperature of human blood serum varied in different diseases. Weltmann noted that the coagulability was affected by the addition of electrolytes before heating. He found that the serum of patients with cirrhosis of the liver was more easily coagulated than was the serum of normal persons, the addition of less electrolyte being required in the former cases than in the latter. On this basis he developed the test which now bears his name.

STANDARD PROCEDURE

0.1 ml. of unhemolyzed blood serum is added to each of ten test tubes containing 5 ml. of varying concentrations of $CaCl_2$ arranged as follows:

Tube No....	1	2	3	4	5	6	7	8	9	10
Conc. %....	0.10	0.09	0.08	0.07	0.06	0.05	0.04	0.03	0.02	0.01

These tubes are then heated in boiling water for fifteen minutes, after which the test is read. The number of tubes in which evident flocculation occurs, rather than mere turbidity, is known as the coagulation band (C.B.). Coagulation occurs more readily in the tubes of higher concentration, normally in the first six tubes (C.B. = 6). Coagulation in less than six tubes is known as a shift to the left or a shortening of the C.B., while coagulation in more than six tubes is known as shift to the right, or a lengthening of the coagulation band.

Weltmann found that a C.B. of 6 was remarkably constant for normal people; that in exudative conditions, such as lobar pneumonia, there was a shift to the left; and that in proliferative conditions, such as chronic fibrotic tuberculosis, there was a shift to the right. Lengthening of the C.B. was noted also in cirrhosis of the liver and in marked congestive heart failure. Since then others have found that there are characteristic changes in parenchymatous liver disease (2) and the test has been used in the study of malignant disease (3) typhoid fever (4) and syphilis (5). It has proved useful in the study of diseases of the kidneys (7) as well as in many other conditions (6, 18).

MECHANISM OF THE WELTMANN REACTION

The underlying mechanism of this test has remained unexplained. Dees (12, 13) has studied the Weltmann reaction clinically and experimentally, and has summarized many of the theories which have been offered to explain the reaction. Chief among these are the theory that the reaction depends upon variations in the pH of the calcium chloride solutions used, the pH of the serum, the serum calcium concentration, the total blood globulin, the albumin-globulin ratio, the blood fibrinogen and the serum lipids. Dees concluded that none of the theories yet advanced is adequate, and suggested that the serum lipids are a decisive factor in determining the length of the coagulation band.

The Weltmann serum coagulation test, as standardized, involves two variables—the concentration of the electrolyte (calcium chloride) and the type of serum used.

In this study certain modifications of the standard technique were made in order to clarify the mechanism of the test.

EXPERIMENTAL VARIATIONS

I. Variation in the Total Quantity of Serum Used

Using the same sample of serum, the standard test in which 0.1 ml. of serum is added to each of the ten tubes of calcium chloride solution was compared with similar series of ten tubes to which 0.025 ml., 0.05 ml., 0.2 ml., and 0.4 ml. of serum was added, respectively. Thus the proportion of serum to calcium chloride was varied through a wide range.

TABLE I

Variations in Total Quantity of Serum Used

AMOUNT OF SERUM ADDED	DILUTION OF SERUM IN $CaCl_2$ SOLUTION	COAGULATION BAND		
		Serum A	Serum B	Serum C
ml.				
0.025	1:200	5	7	7
0.05	1:100	4	6+	7
0.1	1:50	4	6+	6+
0.2	1:25	4	6	6
0.4	1:12.5	4	6	6

TABLE II

Substitution of Other Cations for Calcium in Approximately Equimolar Concentration

SOLUTION	SERUM NO.	TUBE NO.									
		1	2	3	4	5	6	7	8	9	10
$MgCl_2$	1	+	+	+	+	+	+	+	+	0	0
	2	+	+	+	+	+	+	+	+	0	0
	3	+	+	+	+	+	+	0	0	0	0
$BaCl_2$	4	+	+	+	+	+	+	0	0	0	0
	5	+	+	+	+	+	+	+	+	+	0
	6	+	+	+	+	+	+	+	+	0	0

+ Indicates flocculation occurred. 0 indicates no flocculation occurred.

In Table I it is seen that, with the wide variations in the amount of serum used there was almost no variation in the coagulation band. The results indicate that the essential factor in producing the wide variations which may be found in the coagulation band is not the absolute amount of serum, the absolute amount of calcium chloride solution, nor the dilution of the serum in the calcium chloride solution.

II. Substitution of Other Cations for Calcium

Barium chloride and magnesium chloride in approximately equimolar concentration were substituted respectively for the calcium chloride, employing the standard amounts of serum. Thus a series of tubes having approximately the same ionic strength as that of the standard calcium chloride solution was obtained. The variable in this experiment was the cation used.

From Table II it is evident that coagulation occurred more easily in the tubes of higher concentration of barium or magnesium than in the tubes where the electrolyte solutions were more dilute.

DISCUSSION

The fact that coagulation bands form when ions other than calcium are used was also noted by Weltmann in his original article (1). These results indicate that coagulation under the conditions of the Weltmann test is not attributable to a specific property of calcium. The results indicate that there is some change in the character or composition of the serum which is responsible for the variation in the length of the coagulation band in different diseases, and that this coagulation may be produced by ions other than calcium, plus heat.

A new method for studying the composition of the plasma proteins has recently been devised (14). By means of the electrophoretic method of Tiselius the amounts of the different plasma proteins can be determined. Using this method, Luetscher (15, 16), Longsworth, Shedlovsky, and MacInnes (17) and others have described variations in blood proteins in different diseases. In Table III Luetscher's average findings have been arranged in series according to the relative amounts of alpha globulin (Column IV).

It will be seen that the conditions in which high percentage of alpha globulin is found are the same as those in which a short coagulation band occurs and vice versa.

In an effort to gain further information on this point the same specimen of blood was subjected to an electrophoretic analysis and a Weltmann test in 33 instances. These results are shown in Chart I.

It is seen that there is no apparent relationship between the coagulation band and the percentage of the albumin, beta globulin and gamma globulin fractions in the blood serum, respectively. However, it is evident that where the values for alpha globulin were high, the coagula-

TABLE III

Relative Amounts of Plasma Proteins in Different Diseases (15, 16)

I DISEASE	II TOTAL PROTEINS	III ALBUMIN	IV ALPHA GLOBULIN	V BETA GLOBULIN	VI GAMMA GLOBULIN	VII FIBRINOGEN
	gm./100 cc.					
Long coagulation band						
Terminal nephritis	6.3	58.3	5.5	18.7	11.6	5.8
Cirrhosis	5.5	37.8	5.9	22.3	27.9	6.1
Cardiac failure	5.9	45.1	6.5	25.5	17.6	5.2
Normal	6.5	62.5	7.0	13.2	11.6	5.7
Short coagulation band						
Rheumatic fever	5.4	31.1	10.4	16.7	33.5	8.3
Amyloid disease	5.0	26.2	16.7	28.9	28.2	—
Nephrosis	4.0	27.4	15.2	34.2	4.8	18.4

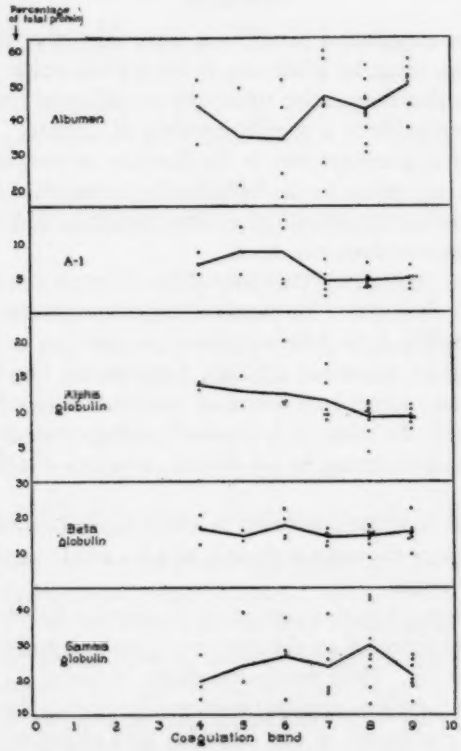

CHART I. RELATIONSHIP BETWEEN THE COMPOSITION OF THE SERUM PROTEIN AND THE COAGULATION BAND

tion bands were short; and where the coagulation bands were long, the values for alpha globulin were low. The curve showing the average determinations illustrates this relationship. Although the range is wide, the tendency is suggestive. It is not implied that this relationship between the length of the coagulation band and the percentage of alpha globulin is a direct one; nor is the Weltmann reaction recommended as a measure of the percentage of alpha globulin in the blood serum. It is the purpose of this preliminary report merely to call attention to this relationship, and to suggest that, while the Weltmann test may depend upon a number of variables in the blood serum, it seems to be associated particularly with the variations in the percentage of the alpha globulin fraction. The Weltmann test should therefore be of value in following the course of certain illnesses in which such variations take place, being useful as a supplement to, and not as a substitute for, the more direct but more difficult electrophoretic determinations.

It would seem desirable to investigate the Takata-Ara reaction, the Hangar cephalin flocculation test, and the formol gel test in a similar manner, since these non-specific tests also vary with liver disease and in certain diseases in which changes in the blood protein fractions are known to occur.

SUMMARY

1. The technique of the Weltmann serum coagulation test and its clinical applications have been briefly described.

2. The mechanism of the reaction has been discussed.

3. It has been shown that the length of the coagulation band depends upon qualitative rather than quantitative changes in the blood serum.

4. Cations other than calcium in equimolar concentration also produce a coagulation band.

5. The inverse relationship between the percentage of alpha globulin in the blood as measured by electrophoretic determinations, and the length of the coagulation band has been demonstrated.

The authors are indebted to Doctor Bernard Davis for the electrophoretic analysis of the blood sera, and to Doctor Caroline Bedell Thomas and Doctor John A. Luetscher for suggestions and criticisms.

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THE SPECIFICITY OF PERIARTERIAL FIBROSIS OF THE SPLEEN IN DISSEMINATED LUPUS ERYTHEMATOSUS

IRWIN H. KAISER

From the Departments of Pathology, the Johns Hopkins University and Hospital and Cornell University Medical College

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The concept of disseminated lupus erythematosus as a generalized disease, rather than as a local process, has grown rapidly in recent years. The disease entity was originally defined by Hebra (1), Cazenave (2) and Kaposi (3). After 23 years, Osler's contributions concerning the erythema group of diseases, which probably included cases of disseminated lupus erythematosus, broke ground for the study of the systemic manifestations of the disorder (4, 5, 6). The next major step was taken by Libman and Sacks (7) two decades later in their description of so-called "atypical verrucous endocarditis," now acknowledged to be one of the manifestations of the disease.

The observation of Libman and Sacks initiated intensive study of the systemic effects of lupus erythematosus, both clinically and postmortem, in the hands of Mook, Weiss and Bromberg (8), Baehr, Klemperer and Schifrin (9), Jarcho (10), Ginzler and Fox (11), Davydovsky (12) and others. The results of the pathological studies have been recently brought up to date and summarized by Klemperer, Pollack and Baehr (13). This work, accomplished in the last 15 years, has made it abundantly clear that the disease is a generalized process, affecting connective tissue throughout the body, whose local manifestations depend on the special response of individual organs and the intensity of the reaction in the individual patient.

The lesions found in the skin, the kidneys and the heart in disseminated lupus erythematosus are now familiar. The changes in the spleen, however, have received little attention.

The earliest reference to periarterial fibrosis of the spleen is that of Libman and Sacks in 1923 (7). Schaumann and Introzzi in 1931 (14) described the lesion carefully in three cases and stated their opinion that disseminated lupus erythematosus was a disease of the hematopoietic system and the spleen. Of the 221 autopsies on patients dying

of the disease found in the literature, but 11 instances of periarterial fibrosis of the spleen had been described prior to the inception of the present study (Libman and Sacks (7) 1923; Füllenbaum (15) 1926; Volk (16) 1928; Schaumann and Introzzi (14) 1931; Belote and Ratner (17) 1936; Denzer and Blumenthal (18) 1937; Ginzler and Fox (11) 1940; Davydovsky (12) 1941). The publication of 19 instances of periarterial fibrosis of the spleen by Klemperer, Pollack and Baehr (13) during the preparation of this report called attention for the first time to the high frequency of the lesion in disseminated lupus erythematosus.

The present study is devoted primarily to the description of periarterial fibrosis of the spleen and to an investigation of its specificity for disseminated lupus erythematosus. The clinical and pathological findings in 18 cases of disseminated lupus erythematosus are summarized and the splenic pathology in these cases is described. The incidence of periarterial fibrosis in sections of spleens from 1679 cases other than disseminated lupus erythematosus is then presented and analyzed. Finally, the question of the specificity of the splenic lesions in disseminated lupus erythematosus is discussed.

MATERIALS AND METHODS

Eighteen cases of disseminated lupus erythematosus have been included in the group from which material for this study was obtained. Seven of these are from the files of the Department of Pathology of the New York Hospital (#8460, #9096, #9392, #9561, #9744, #9750 and #9935). The remaining eleven form the complete series of recognized cases which have come to autopsy in the Department of Pathology of the Johns Hopkins Hospital since 1922 (#7486, #11465, #12961, #14514, #15996, #16393, #16554, #17051, #17346, #17378 and #17440). The first six of these latter cases have been reported in detail by Keefer and Felty (19), Jarcho (10) and Maumenee (20).

Sections forming the control series were obtained in the files of the Johns Hopkins Hospital Department of Pathology.

Elastic tissue stains were made by the method of Verhoef (22), and reticulum stains by minor modifications of Foot's modification of the Hortega silver ammonium carbonate technique (23).

Measurements of the follicular arteries were carried out with a calibrated eyepiece micrometer and represent the average of measurements of ten such arteries.

SUMMARY OF CASES OF DISSEMINATED LUPUS ERYTHEMATOSUS

Of the 18 cases of disseminated lupus erythematosus there were 14 females and 4 males. Three of the females were Negro and the other 15 patients were white. The youngest was a girl of 10 years, the oldest a man of 65. All the other cases fell between 18 and 45 years of age. At some time or other every patient manifested an erythematous skin eruption. In 15 there was joint involvement, and in a similar number pleurisy had occurred. Blood studies revealed that 17 had anemia at some time, while 9 had definite leukopenia. Several others were noted to have normal white blood cell counts in the presence of severe infection. Three patients had marked thrombocytopenia. Nine were found to have clinical evidence of kidney damage. Two were reported to have a positive serologic test for syphilis.

In at least 10 cases, death resulted from overwhelming infection, usually bronchopneumonia. There was one death from cardiac failure and there were several from "toxemia." One patient died of miliary tuberculosis. Post-mortem evidence of the disease included the finding of skin lesions in a majority of cases and multiple involvement of the serous cavities in at least 14 instances. Verrucous endocarditis was observed 5 times, and the "wire-loop" glomerular lesion (Baehr, Klemperer and Schifrin (9); Stickney and Keith (21)) of the kidney in 4 cases. Periarterial fibrosis of the spleen was seen 15 times. In no case were the pathological findings inconsistent with the clinical diagnosis.

DESCRIPTION OF THE SPLENIC LESIONS

The most frequently encountered gross lesions was a perisplenitis, found in 10 cases of this series of 18. In two cases this took the form of a fresh fibrinous exudate over the capsule, while in one of these and in eight others, fibrous bands or adhesions to liver, diaphragm or omentum were noted. The occurrence of this lesion and its characteristics bore no apparent relationship to the intensity or duration of the disease process.

The spleen was usually definitely but not markedly enlarged. The average weight was 262 gms., but one-third of the cases fell near or within the limits of normal size. Enlargement of the spleen was found in the presence of terminal infection.

Evidence of tuberculosis was noted in four cases, in three of which solitary healed tubercles were found. In the fourth, however, miliary distribution of tubercles with caseation and necrosis, associated with generalized miliary tuberculosis, was encountered.

Microscopically, periarterial fibrosis was found in 15 of the 18 cases. For the purposes of this study, periarterial fibrosis was considered present in the spleen when the periarterial collagen of the penicillary or follicular arteries, which normally is closely packed and without evidence of hyalinization, was found to be present in at least three layers, around at least half the circumference of the vessels, producing the appearance of concentric rings. In no case was periarterial fibrosis found in relation to the medium or large arteries or about any of the venous structures. In most cases this collagen was hyaline and in some of these it was partially broken down to granular eosinophilic material. The lesion was found in a few cases around only two or three penicillary vessels, but in most instances the majority of the smaller arteries was affected. Where the change was most extensive, all the penicillary arteries and more than half the follicular arteries were surrounded by this hyaline collagen.

No early or intermediate stages of the lesion were observed. When stained for reticulum, several layers of collagen were present around affected vessels. No reticulum was found among them, although the collagen was continuous with the follicular reticulum, which itself was unaltered. The few cells present appeared to be fibroblasts and in no cases were there the cells of inflammation. In a few of the cases with acute splenic tumor, there was the round cell infiltration of the external perivascular collagenous connective tissue which is occasionally observed in that condition. In one instance there was extreme hyaline degeneration of the fibrotic collagenous fibers. These remained only as small, deeply eosinophilic masses which suggested the ringed arrangement.

As a consequence of the fibrosis, the width of the follicular vessels was increased, the average diameter in affected cases being about 125

μ in contrast to the normal average of 60 to 75 μ. This, despite a consistent increase in the size of the lumina, resulted in a definite increase of the wall:lumen ratio.

In association with these alterations, there did not appear to be any change in the inner coats of the arteries. In five cases of this series there were hyaline changes in the media of the type frequently observed in the spleen in other conditions, three of these being in the same sections as the periarterial fibrosis. The elastic tissue stain failed to disclose any changes in this substance.

Other changes such as acute splenic tumor, hemorrhage into the pulp, and focal necroses, were observed infrequently and in association with complications of the disease process which fully accounted for their presence.

CONTROL SERIES

The spleen sections from 970 consecutive routine autopsies, exclusive of those in the selected series and exclusive of cases under 10 years of age, were studied microscopically for vascular alterations. Periarterial fibrosis, as defined above, was found in 22 cases, an incidence of 2.3% (see Table I). In the majority of these, the fibrosis was found around only a few penicillary arteries, but in a considerable number the change was widespread. In no instance, however, was it as extensive, either in size or in distribution, as in disseminated lupus erythematosus.

In addition, 709 other cases were studied. These were selected for clinical or pathological changes in systems which disseminated lupus erythematosus is known to attack. These are listed in Table II. 31 instances of periarterial fibrosis were encountered in this group, an incidence of 4.4% which is not significantly different from the incidence of 2.3% in the series of consecutive unselected cases. In the majority of these cases, the lesion was limited in distribution, either moderately affecting a few penicillary arteries or slightly altering many of them along with the follicular vessels.

It may be seen that in 6 groups of selected cases, an incidence greater than that of the control series was observed. In the cases of arteriosclerosis, rheumatic fever, acute splenic tumor, chronic passive congestion and periarteritis nodosa, the differences are not statistically significant. Nevertheless, in each case in these groups in which peri-

arterial fibrosis was encountered, the clinical history was reviewed, and in only one were there grounds for a suspicion of disseminated lupus erythematosus. This patient, a white male of 37 years, had had an erythematous skin eruption, regarded by dermatologic consultants

TABLE I

Incidence of Periarterial Fibrosis of the Spleen in 1679 Control Cases

	NUMBER	PERI-ARTERIAL FIBROSIS	PER CENT
Series of consecutive routine autopsies	970	22	2.3
Series of cases selected for special clinical or pathological features	709	31	4.4
Total	1679	53	3.2

TABLE II

Anatomical Diagnoses and Incidence of Periarterial Fibrosis in 709 Selected Cases

DIAGNOSIS	NUMBER	PERI-ARTERIAL FIBROSIS	PER CENT
Arterio- and arteriolosclerosis, general	235	11	4.7
Rheumatic fever, acute and chronic	134	9	6.7
Tuberculosis, miliary, disseminated or healed tubercle of the spleen	111	2	1.8
Acute splenic tumor	100	4	4.0
Chronic passive congestion of the spleen	90	7	7.7
Leukemia, all types	57	0	0.0
Anemia, all types	52	0	0.0
Periarteritis nodosa	15	1	6.6
Essential thrombocytopenic purpura	13	4	30.8
Miscellaneous	74	0	0.0

N.B.: Many of the sections are listed under more than one Anatomical Diagnosis and the total is therefore greater than the number of cases. This is likewise true of the instances of periarterial fibrosis.

as unlike that of disseminated lupus erythematosus, as well as mild fever, a palpable spleen, hypertension, severe muscle pains and evidence of renal damage. The clinical diagnosis of periarteritis nodosa was confirmed at autopsy.

In the group of cases of essential thrombocytopenic purpura, the finding of periarterial fibrosis in 4 cases out of 13, or 31%, was statisti-

cally significant as compared with the consecutive control series. In all four cases the clinical history was reviewed and found to be entirely typical of essential thrombocytopenic purpura. Further, the usual clinical features of disseminated lupus erythematosus such as the skin eruption, polyarthritis, pleural effusion, fever, leukopenia and renal damage were all lacking. In the two cases in which splenectomy was performed, there was the expected post-operative response clinically, proven by study of the blood, and the patients were discharged from the hospital improved. The other two patients died of severe hemorrhage, and complete autopsy failed to reveal any of the recognized stigmata of disseminated lupus erythematosus in the skin, cardiovascular system or kidneys. In one case in which splenectomy was performed and in another which came to autopsy, the periarterial fibrosis was marked both in extent and distribution. These are shown in Figures 2 and 3.

Marked periarterial fibrosis was found in four other cases. One was that of a female of 41 years who died as a result of septicemia following an infected abortion. There was post-mortem evidence of scarred rheumatic endocarditis and arteriosclerosis. Another was a male 32 years of age, with a typical past history of acute rheumatic fever, who died of cardiac failure due to extreme rheumatic valvular damage. The third was that of a male of 72 years who died of pyelonephritis and marked arteriosclerosis. The final instance was that of a female of 19 years who died of an exacerbation of acute rheumatic fever. At autopsy she was found to have rheumatic involvement of the left auricle and extensive scars of Aschoff bodies in the myocardium. Complete autopsy in these cases did not reveal positive evidence of disseminated lupus erythematosus, nor was there any evidence of the existence of that disease in the clinical histories.

In summary, the incidence of periarterial fibrosis in the entire series of 1679 control cases was 53 cases, or 3.2%.

DISCUSSION

The most striking lesion observed in the spleen in disseminated lupus erythematosus, namely periarterial fibrosis, presents only minor variations in its morphology. The collagen fibers are separated and have a ringed appearance which has been characterized as "onion-peel." Around the penicillary arteries the layers of collagen are usually greater

in number than in the case of the follicular arteries. Varying numbers of fibroblasts may be present, but inflammatory cells are not observed. Periarterial reticulum is essentially unaltered. The intima, elastica and media apparently do not undergo changes in association with these periarterial alterations. In most cases in this series, the collagen apparently underwent hyaline change, leaving in some areas fine granular eosinophilic material in continuity with the fibrotic fibers.

The frequency of the periarterial fibrosis in the smaller arteries varies from case to case. In one, but a few penicillary arteries may present the change, with only a few of the follicular vessels altered. In another, virtually every one of the smaller arteries may be strikingly affected. All stages between these two extremes may be encountered. However, as has been stated, no early or intermediate stages of the lesion are seen; the collagenous fibers either are or are not present and no necrotic or inflammatory changes are seen.

In considering these morphologic characteristics, it is difficult to arrive at any decision about the developmental stages of periarterial fibrosis. The early stages appear to represent but a slight degree of the same process. No preliminary changes of either the reticulum or the collagen which could be construed as setting the stage for subsequent formation of the well-developed collagenous connective tissue fibers were observed. Whether some transitory acute damage of this connective tissue occurs which does not persist sufficiently long to be observed at autopsy cannot be determined. In any event, there appears to be no definite connection between the post-mortem occurrence of periarterial fibrosis and any aspect of the clinical course.

The connection between the periarterial fibrosis and the other lesions of disseminated lupus erythematosus is obscure. Verrucous endocarditis, the so-called wire-loop lesion in the kidney and the widespread changes in the arterial system represent a type of connective tissue damage which especially affects the collagen. These lesions, while they are not specific for disseminated lupus erythematosus, nevertheless are known to occur with a high frequency in this disease. The periarterial fibrosis in the spleen is morphologically quite different from these changes. To be sure, the alteration is one of the collagenous connective tissue, as Klemperer, Pollack and Baehr (13) have emphasized. But there is no evidence of inflammation or necrosis. Further, there are no developmental stages observed; the lesion, which

has all the appearances of an old burned out process, either is or is not present. This is in contrast to the cardiac and other vascular manifestations of disseminated lupus erythematosus.

The question of the specificity of the splenic lesions of disseminated lupus erythematosus has been raised recently by Keil (24) and Montgomery (25), although neither states specifically that periarterial fibrosis has been found in other disease processes.

Reactions in the spleen to complications of the fundamental disease process, such as acute splenic tumor, hyperplasia, infarcts, chronic passive congestion and focal necroses are certainly not specific. Further, there are no recognized characteristics of the perisplenitis found in disseminated lupus erythematosus which distinguish it from that found in other inflammatory conditions of the peritoneal serosa.

Klemperer, Pollack and Baehr in their paper on the pathology of disseminated lupus erythematosus (13) have stated that "the peculiar periarterial sclerosis found in nearly every case is so arresting that it must be considered specific. We have not encountered a fully developed similar lesion in a wide variety of control cases." However, a lesion cannot be satisfactorily defined in its most advanced and striking form. The occurrence of splenic periarterial fibrosis in a solitary vessel in a section is evidence that damage of this type has occurred in many thousands of instances throughout the organ. The lesion must therefore be defined in its minimal form.

In the 1679 control cases examined in this study, periarterial fibrosis as herein defined was found in 53, an incidence of 3.2%. Further, there were at least 6 instances in which the lesion was "fully developed" and could not be distinguished from the most markedly affected cases of disseminated lupus erythematosus. There are at present no known morphologic characteristics by which periarterial fibrosis as it may occur in isolated vessels or generally distributed in the spleen in other conditions can be distinguished from even the advanced instances associated with disseminated lupus erythematosus.

Because of the small number of cases available, it is not possible to state definitely that periarterial fibrosis in the spleen is a frequent finding in essential thrombocytopenic purpura. In the present series, the lesion did occur in that disease to a highly significant extent in comparison with its incidence in the remainder of the control series. A final decision must await further study.

However, it is clear that periarterial fibrosis in the spleen does occur with a high frequency in disseminated lupus erythematosus: 15 out of 18 cases of the present series. Its discovery post mortem should at least raise the suspicion of that diagnosis and encourage study both clinical and pathological for other stigmata of the disease. Further, its coincidence with the other well recognized lesions of the connective tissue such as verrucous endocarditis and the "wire-loop" glomerular changes can serve to strengthen the post-mortem diagnosis of disseminated lupus erythematosus. The lesion cannot, however, in the light of our present information, be considered pathognomonic of disseminated lupus erythematosus, regardless of its extent in any given case.

SUMMARY

1. The splenic pathology in 18 cases of disseminated lupus erythematosus is summarized. Perisplenitis and periarterial fibrosis were the commonly observed lesions. Periarterial fibrosis was found in 15 of the 18 cases in this series.

2. The perisplenitis presents no characteristics by which it may be differentiated from that commonly seen in other conditions.

3. Periarterial fibrosis is defined herein as the occurrence of at least three separated layers of the normally densely packed periarterial collagen of penicillary and follicular arteries, in such a manner as to give the typical ringed appearance. The collagen is frequently hyaline and granular eosinophilic material may be found continuous with it. The process apparently is an alteration of collagen independent of necrosis or inflammation. No early or intermediate stages have been observed.

4. In a control series of 1679 splenic sections periarterial fibrosis occurred in 53 cases, an incidence of 3.2%. No evidence of the occurrence of disseminated lupus erythematosus could be found in any of these cases.

Periarterial fibrosis occurred in 4 cases of essential thrombocytopenic purpura out of 13 examined, an incidence of 31%. This is the only group besides the cases of disseminated lupus erythematosus which significantly differed from the control series.

5. The splenic lesions of disseminated lupus erythematosus are not pathognomonic of that disease, although periarterial fibrosis occurs

in a very high proportion of the cases. There are no known characteristics of the splenic periarterial fibrosis found in disseminated lupus erythematosus which will distinguish it from that found in other conditions. In view of its frequent occurrence in disseminated lupus erythematosus, however the discovery of the lesion at autopsy should raise a suspicion of disseminated lupus erythematosus and provide impetus for further investigation. When found in association with the other familiar stigmata of disseminated lupus erythematosus periarterial fibrosis in the spleen provides corroborative evidence.

The author wishes to thank Dr. Jacob Furth, Dr. Arnold R. Rich and Dr. Ella H. Oppenheimer for guidance in the preparation of this report; Miss Sylvia Sigelman for the elastic tissue stains; Mrs. Regina Plavsky of the Army Medical Library for assistance in translation; and Mr. Milton Kougl for the accompanying microphotographs. He also wishes to thank his wife for unflagging encouragement and ever-willing help in all phases of the work.

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PLATE I

Fig. 1. Photomicrograph (× 90) of spleen from a case of disseminated lupus erythematosus in a white male of 19 years who died of bronchopneumonia and cachexia (J.H.H. #17346). Periarterial fibrosis was marked in this case and is shown here surrounding three follicular arteries.

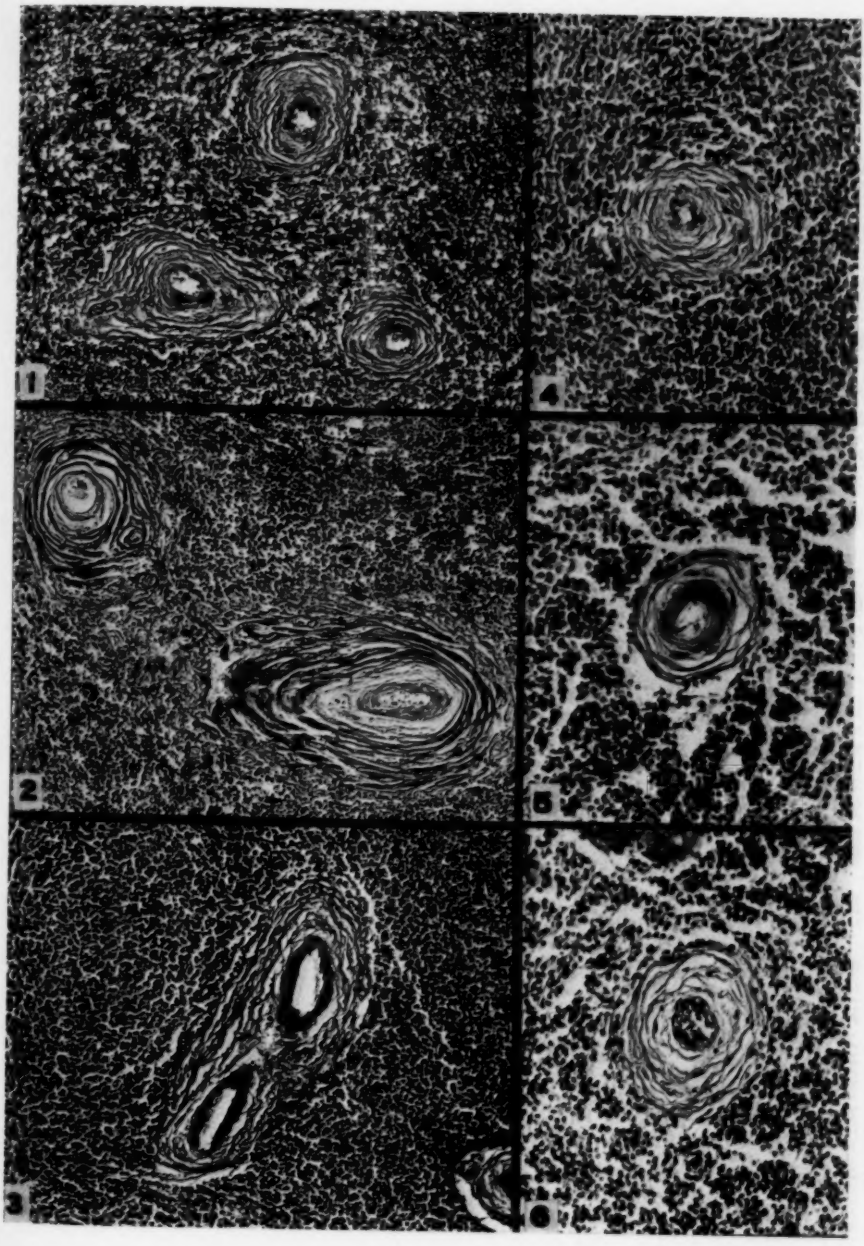
Fig. 2. Photomicrograph (× 90) of spleen from a case of essential thrombocytopenic purpura in a Negress of 22 years who died as a result of severe hemorrhage (J.H.H. #10834). The very marked periarterial fibrosis found in this case is shown in two small vessels.

Fig. 3. Photomicrograph (× 90) of spleen from a case of essential thrombocytopenic purpura in a white female of 26 years on whom splenectomy was successfully performed (J.H.H. Surg. Path. #78643). Periarterial fibrosis as seen here in two follicular arteries was found in several areas in the section.

Fig. 4. Photomicrograph (× 180) of spleen from a white female 19 years of age who died of cardiac failure and who was found to have chronic rheumatic endocarditis affecting the tricuspid, mitral and aortic valves and left auricle (J.H.H. #15889). Periarterial fibrosis of this type was found throughout the section, affecting the follicular arteries primarily.

Fig. 5. Photomicrograph (× 180) of spleen from a Negress of 39 years who had hypertension, arteriosclerosis, nephritis and anemia and who died in cardiac failure (J.H.H. #13240). This shows almost minimal changes of periarterial fibrosis, observed in only an occasional vessel in the section, as well as the frequently seen medial hyalinization.

Fig. 6. Photomicrograph (× 180) of spleen from a pregnant white female 20 years of age who died without explanation at term. Atrophy of the liver, pulmonary oedema and cloudy swelling of the kidney were found (J.H.H. #13330). The periarterial fibrosis here is well marked and of the type occasionally found in a solitary vessel in the section.



UNIV. OF MICH.

BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal.)

Diagnosis and Treatment of Diseases of the Heart. By HENRY A. CHRISTIAN. 599 pp. $7.00. *Oxford University Press, New York [etc.], 1940.* (Reprinted from Oxford Monographs on Diagnosis and Treatment.)

The book is one of the Oxford Monographs on diagnosis and treatment. The author makes no claim to its being a complete textbook of diseases of the heart. Rather, it is designed to guide practitioners of medicine in dealing properly with patients suffering from heart disease. The author presupposes a general knowledge of medicine and there is no extensive discussion of etiology, pathology, physiology, and prognosis. It is unusually complete and reflects the extraordinarily wide clinical experience and general interest of the author. There is a full discussion of the pharmacological action of digitalis drugs. The reviewer believes that the book will be a valuable addition to the libraries of practitioners of medicine.

B. M. B., JR.

America's Last King. By MANFRED S. GUTTMACHER. 426 pp. Illus. $3.50. *Charles Scribner's Sons, New York, 1941.*

The popular reception of this fascinating biography has already indicated how welcome to intelligent readers is the straightforward and detailed account of a patient's life when that life and that illness have great political and historical significance. The author's psychiatric colleagues have also been charmed by this unpretentious yet thorough presentation of a case study so remarkably free of gratuitous psychologizing. For readers, medical or non-medical, here is a warmly human document, sympathetic and kindly, yet objectively candid in its treatment of the individual, and especially interesting for its portrayal of the psychiatric ideology and skill of a period important as a turning point in psychiatric history.

J. C. W.

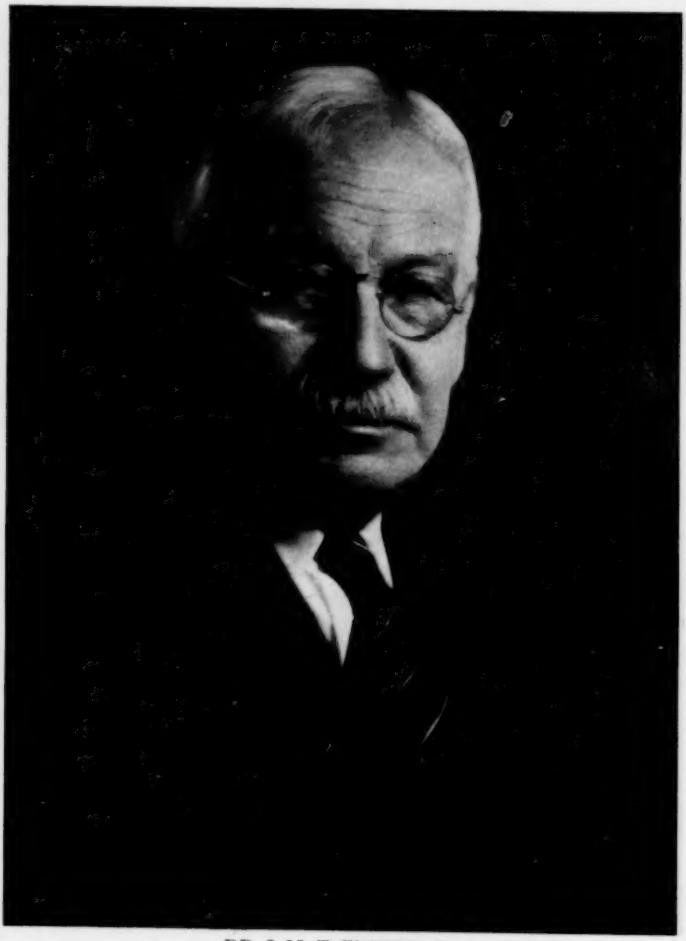
Dr. J. M. T. Finney, Sr.

MINUTE ADOPTED BY THE MEDICAL BOARD, JUNE 29, 1942

With the death of Dr. John M. T. Finney on May 30, 1942, there passed out of the life of Baltimore one of its most eminent citizens, a distinguished surgeon and a man beloved by all who knew him. Though Dr. Finney was born in Natchez, Mississippi, on June 20, 1863, almost seventy-nine years ago, was educated at Princeton College, received his medical degree from Harvard at the age of 26, and his internship at the Massachusetts General Hospital, he will always be identified with Baltimore, for it was at The Johns Hopkins Hospital and Medical School that he developed his remarkable surgical skill, and attained professional eminence; and it was to Baltimore's civic institutions and organizations that he contributed so generously of his time and thought.

He came from the Massachusetts General Hospital to Baltimore in 1889 as an Associate in Surgery on the staff of The Johns Hopkins Hospital. The years following his arrival were devoted entirely to the clinic and to teaching, but as his reputation grew, demands upon his time multiplied. He became in succession Associate Professor of Surgery, Professor of Clinical Surgery and later Acting Professor of Surgery in The Johns Hopkins University; as well as Visiting Surgeon and for a period, Surgeon-in-Chief to The Johns Hopkins Hospital. Early in his career he became an active member of many national societies and was at various times President of the American Surgical Association; the Southern Surgical and Gynecological Association; the Medical and Chirurgical Faculty of Maryland and the American College of Surgeons of which he was a founder. Honorary degrees from many institutions in this country and abroad were conferred upon him and he held an Honorary Fellowship in the Royal College of Surgeons of England and the Royal College of Surgeons of Ireland.

Undoubtedly the years spent in the Army during the last war were important ones for Dr. Finney. He often spoke of them and cherished in his memory associations that he made at that time. With the commission of Major he went to France as Director of Base Hospital



DR. J. M. T. FINNEY, SR.

18, but it was not long before his valuable services were required for more extensive work in the American Expeditionary Forces. The important post of Chief Consultant was given him and shortly afterwards he received the rank of Brigadier General. For his outstanding service to the Armies he was awarded the Distinguished Service Medal, decorated with the Insignia of an officer of the French Legion of Honor, and made a Commandeur de l'Ordre de la Couronne of Belgium.

Though Dr. Finney had always been interested and helpful in matters concerning the welfare of human beings, he became more engrossed than ever, after his return from France, in civic affairs. Previously he had been on the School Board of Baltimore City, from which he had resigned, but now he was appointed a member of the State Board of Education. He was deeply interested in the education of boys, and was President of the Boards of Trustees of both the Gilman Country School and the McDonogh School; a trustee of Princeton University and of Lincoln University. Indeed the extent and breadth of his interests and activities were astounding. Director of the Red Cross, Chairman of the Community Fund, and elder of the Presbyterian Church of the United States were among some of the other important responsibilities that were placed upon his shoulders—responsibilities which he took seriously and to which he gave much, for it was characteristic of Dr. Finney that nothing could induce him to accept a position, however flattering the offer, unless he considered himself fitted for the work. He thus set aside among many other requests a suggestion that he run for senator, and refused the presidency of a university.

Few men have led such completely full and useful lives as Dr. Finney; or have accomplished so much of practical value in so many fields of human endeavor. As a doctor and a man, his patients and friends placed unbounded confidence in him, and as a surgeon his associates and confreres accorded him preeminence in the profession. Though absorbed in the practical problems of life, he was himself of all men the most completely unworldly. He was loved and revered by all who knew him for his humor and his high sense of honor, for his superb surgical skill, for his sympathetic understanding of the needs of others without respect to race, creed or color, and for his instanta-

neous response to a call for assistance. Of Dr. Finney it can be truly said that he experienced great satisfaction from rendering aid to others and happiness in relieving their ills and ministering to their wants. His work, now finished, was done not as a task but with so much exuberance and in such a spirit of real joy that the way of his living will remain as a bright example for years to come.

THE USE OF T-1824 IN PLASMA VOLUME DETERMINATIONS

PHILIP B. PRICE AND WILLIAM P. LONGMIRE

From the Department of Surgery, Johns Hopkins University

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The dye-dilution method of measuring plasma volume, though greatly improved since its introduction in 1915, is still an object of criticism, and many investigators question its reliability. The authors are among those who have not been able to obtain altogether satisfactory results with the test as it is customarily performed. The problem of blood volume is so important, however, that it warrants continued efforts to increase the accuracy of the test or else to find an acceptable substitute for it.

Theoretical considerations persuaded us that the dye-dilution method is sound in principle, and that the difficulty with it may have been due in the main to faulty technique or misinterpretation of results. A critical study of the test revealed that the procedure, in our hands at any rate, is liable to a great many technical errors, and that we had in fact been misinterpreting our results because of inadequate understanding of the behavior of T-1824 in the body and the significance of concentrations of dye in plasma. These observations led us to modify the test in several respects. The revised procedure has been given a thorough trial in the course of an investigation of experimental shock. As a result we believe that we can measure plasma volume in dogs more accurately than heretofore, and that we can follow fairly closely the variations in plasma volume which take place in the development of shock and in recovery from shock.

In this paper we shall review the dye-dilution method, point out certain potential errors inherent in the older techniques and procedures which may be used to correct them, describe our modifications of the test, and discuss the interpretation of results. Our adaptation of the method to clinical investigation will be described in subsequent papers.

1. GENERAL PRINCIPLES

The method as originally described (1) was simple in principle: a known amount of a vital red dye was injected intravenously; after a few minutes allowed for mixing, a sample of plasma was obtained and this was examined with a simple colorimeter against standards containing known amounts of dye; from these data plasma volume was calculated. There was difficulty, however, in distinguishing the red color of the dye and the natural color of serum, especially in the presence of hemolysis. This obstacle was overcome in part by substituting T-1824, a non-toxic, slowly diffusing, blue dye (2). Even so, the results were inaccurate because of incomplete mixing of dye and blood, elimination of some of the dye during the mixing period, and the presence of varying amounts of natural coloring matter in plasma. Smith (3) improved the preparation of the standard solutions and suggested making repeated determinations of blood volume with multiple injections of dye. Gregersen (4) has estimated the error in these older methods to be as much as 30 per cent. He and his associates (5, 6, 7, 8) greatly increased the accuracy of the test by extending the time allowed for mixing to 20 minutes or longer, by measuring dye dilution selectively with a spectrophotometer in absolute terms of optical density, and by plotting a time-concentration curve from a series of blood samples. Gregersen projected this curve back to the time of injection to obtain a value which "is assumed to represent dye-concentration which would exist if the dye were uniformly distributed in the plasma and none of it had been lost from the circulation." Gibson and Evelyn (9) adapted the method to a photoelectric colorimeter and suggested a formula to correct for hemolysis. Gibson and Evans (10) proposed an "indirect method" of following changes in plasma volume during an experimental period.

Manifestly, some of the gross errors in the original method have been corrected by these later refinements and its usefulness has been extended. Many inherent and potential errors remain, however, as the following analysis will show.

2. INJECTION OF DYE

Measurements by weight show that volumetric injections of dye solution with the calibrated 10 cc. syringe recommended by Gibson and Evans (9) are liable to errors as large as $\pm$ 0.1 gm. because of

inability to set the plunger exactly, different degrees of filling and emptying the needle, and variations in seepage of the solution around the plunger. It has been found also that variable amounts of dye are always left in the syringe and in the hub of the needle even after repeated rinsings with venous blood. Furthermore, dissection of the site of injection shows that in a good many cases some of the concentrated dye solution has been injected, or has escaped, into the wall of the vessel or into the adjacent tissues. Though easily overlooked, such errors are significant; they may be gross if small amounts of dye are used in the injections.

To avoid these possible errors we have adopted the following routine in dogs: (1) A glass-tipped 5 cc. Luer syringe and snugly fitting 22 gauge needle are loaded with the approximate amount of dye solution to be injected. (2) Syringe, needle, and solution are weighed on scales having an accuracy of less than ± 5 mgm. (3) An incision is made exposing the femoral vein where it lies superficially in the femoral triangle. (4) The needle is thrust, pointing proximally, a distance of a centimeter or more into the lumen of the vessel. If properly placed, the point can be seen through the thin venous wall and the injection is literally made under direct observation. The plunger is held down to prevent any blood from entering the needle, which is left in place for a full minute before withdrawal. Light pressure with a sponge for a few moments prevents bleeding from the needle hole. The skin incision may be closed temporarily with a clip so that the same vein can be used for subsequent dye injections. Should there be any leakage of dye at all during this procedure the injection is halted forthwith, and the entire performance is repeated after 20 minutes or longer, a "dyed dye-free" specimen being taken immediately prior to the re-injection. (5) The outsides of the needle and syringe are wiped clean and dry and are weighed without delay. The difference in weights before and after injections represents the weight of dye solution given, and from this weight the volume of dye solution and the amount of dye injected can be readily calculated. The intrinsic error in this procedure is extremely small.

3. PLASMA SAMPLES

Theoretically, three variables disturb the homogeneity of dye distribution in the circulation: Removal of dye from blood as it passes

through the liver, flow of streams of less deeply stained lymph into the great veins at the base of the neck, and regional differences in capillary permeability to the dye. Although we have not been able to demonstrate significant differences in concentration of dye in blood taken synchronously from arteries, superficial leg veins, jugular vein, and right auricle in normal dogs, arterial samples theoretically represent average dye concentrations more accurately than venous samples. The method which we have used to obtain repeated samples of arterial blood has been described elsewhere (12).

Large errors may arise from the use of anticoagulants. Heparin colors the plasma sufficiently to interfere with dye determinations (Gregersen and Schiro (7)). If isotonic anticoagulant solutions[1] are used, homogenous mixing with the blood sample must be assured, and correction for dilution of the plasma must be made on the questionable basis of comparative hematocrit determinations. Dry sodium oxalate causes considerable shrinkage of red cells and corresponding dilution of the plasma. Large errors may be introduced in this way. These difficulties may be avoided by using serum instead of plasma. Gregersen and Schiro (7) have shown that coagulation does not reduce the amount of dye in solution. Serum has certain disadvantages, however: The samples are technically more difficult to prepare; troublesome or ruinous hemolysis may occur (especially in working with canine blood); and the material cannot be used for other routine determinations, such as hematocrit and total plasma proteins.

We have found it satisfactory in many experiments to employ a mixture of dry ammonium and potassium oxalate in the proportion of 13 to 7, which has been shown (Wintrobe *et al.* (14)) to have minimal effects upon corpuscular volume. We use about 4 mgm. of the oxalate salts per cubic centimeter of plasma. The mixture is prepared in solution (1.3 gm. ammonium oxalate, 0.7 gm. potassium oxalate, 100 cc. distilled water), 0.5 cc. of which is put into each specimen bottle and dried at about 50 C. Knowing the approximate cell/plasma ratio of the circulating blood at the time the specimen is taken, we put that amount of blood into the bottle which will yield an estimated 2.5 cc.

[1] Hooper *et al.* (13) found 2 cc. of 1.6 per cent sodium oxalate solution in 9 or 10 cc. of blood to be isotonic. Gregersen and Schiro (7) recommend 1.5 per cent sodium oxalate solution, using 1 part of oxalate and about 4 parts of blood (20).

of plasma. One cc. or more of this oxalated blood is taken for hematocrit determination, cell counts, and the like, and the remainder is centrifuged, the plasma being transferred without delay to small stoppered test tubes for subsequent examination with the colorimeter. These samples of plasma are clear and show minimal degrees of hemolysis. We believe that they represent fairly accurately the plasma of the circulation, and what error of dilution may be present is more or less uniform in all the specimens. This last consideration is important since our measurements of dye are relative rather than absolute.

4. MEASUREMENT OF DYE CONCENTRATION

An advantage of the photoelectric colorimeter is that it eliminates the personal equation involved in matching or measuring color intensities optically; instead, amounts of light of selected wave lengths passing through layers of plasma of standard thickness are recorded by a galvanometer.[2] Two filters are recommended, number 620 (maximum transmission at 620 millimicrons) for the blue dye, and number 540 M (maximum transmission at 540 millimicrons) for hemoglobin. Galvanometer readings, G, are converted into so-called L values which are simply 2-log G. These L values, representing average optical densities over the narrow band of wave lengths, have a linear relationship to concentration of color in the solutions (Bier's law). In these tests the L values represent relative rather than absolute measurements of optical density. That is to say, a sample of dye-free plasma (or serum) is placed in the machine and, with the proper filter, an intensity of light is employed which gives a galvanometer reading of 100; then, with exactly the same amount of light, dyed samples are read. When dye is re-injected, Gibson and his associates use a sample taken immediately prior to the re-injection for the standard, setting the galvanometer at 100 for it. They speak of this as a "dyed dye-free" sample. Gibson and Evelyn have proposed formulas, (a) to estimate plasma volume, and (b) to correct for hemolysis.

[2] The following discussion refers to the Evelyn Photoelectric Microcolorimeter (11), manufactured by the Rubicon Company, Philadelphia, and its use in plasma volume determinations with T-1824, as described by Gibson and Evelyn (9). We have used this apparatus exclusively in the present investigation.

(a) $$\text{plasma volume} = \frac{\text{mgm. dye injected}}{L_{620}\text{ (extrapolated)}} \times K\text{ (a calibration constant)}$$

(b) $$L_{620}\text{ corrected} = \frac{(20 \times \overset{\prime}{L}_{620}\text{ as read}) - L_{540M}}{20.0 - 0.6}$$

The precision of the apparatus and the limits of accuracy of the procedure just described have been estimated. The constant K in formula (a) must be determined for each new lot of dye solution. In one set of tests Gibson and Evelyn (9) found variations in individual standardizations to be ± 1.5 per cent. Comparing the photoelectric method with that employing the spectrophotometer, the same authors found the former to be accurate within ± 2.5 per cent. That probably is an unduly conservative estimate. In a large series of duplicate determinations we were always able to repeat dye readings within ± 1.25 per cent, and usually within 1 per cent. These represent intrinsic errors which should be kept to a minimum by meticulous technical accuracy and attention to detail. An error of 1 or 2 per cent in the final calculation of plasma volume might well be considered negligible, but the errors just mentioned affect individual dye readings which are used to establish and project the curve of dye disappearance. The further this curve is projected, the greater the possible magnification of these errors.

Formula (b) is based on the observation (9) that the ratio $L_{540\,M}:L_{620}$ for hemoglobin is 20; for the dye, 0.6. One of us (W. L. P.), likewise using an Evelyn Photoelectric Micro-colorimeter and a quantity of T-1824 purchased from the Eastman Kodak Company, obtained ratios of 22.7 and 0.53. Subsequently, the senior author, using another machine and set of filters and a lot of very pure dye kindly supplied by Dr. Gergersen, obtained ratios of 19.8 and 0.645. Substitution of these ratios in formula (b) gives virtually the same answer in slightly hemolyzed specimens, but different correction values in the case of deeply hemolyzed specimens such as may be found in dogs suffering from severe burns. It would appear advisable, therefore, to determine the proper $L_{540\,M}:L_{620}$ ratios for each machine and each lot of dye.

Gibson and Evelyn (10) recommend correction for hemoglobin in all hemolyzed samples, but offer no criteria for degree of hemolysis requiring correction. Routine examination with the 540 M filter re-

veals that many samples which to the eye appear non-hemolyzed nevertheless contain sufficient hemoglobin to alter the dye readings significantly. The more deeply dyed specimens in particular are apt to mask hemolysis. In the interest of accuracy we have applied formula (b) to all samples that contain dye.

Postprandial lipemia interferes with accurate reading of dye concentration mainly because samples of plasma contain progressively decreasing amounts of fat but are all read against a standard which, having been taken earlier, contains a relatively large amount of fat. Since the photoelectric colorimeter does not distinguish between optical densities caused by dye and by colorless fatty particles, dye readings may be unduly low, especially in those samples taken some time after the standard was obtained. It is important, therefore, that food should be withheld from the subject for at least 12 hours before the test is begun.

5. MIXING TIME

It was observed by those who first used the dye-dilution method (1, 2, 3) that within two or three minutes after injection the dye is diluted by the blood to a degree which remains unchanged for the ensuing four to six minutes. Consequently, those investigators took samples of blood four minutes after injection of dye in the belief that mixing was complete at that time and that the dye had not yet begun to disappear. A different opinion is held by Gregersen and Stewart (8), and by Gibson and Evans (9), who studied curves of dye disappearance and concluded that from 8 to 20 minutes are required for complete mixing in normal individuals and even longer periods in persons with abnormally slow circulation. On the other hand, Gilder, Muller and Philips (15) hold that full mixing requires less than five minutes. This view is based on the observation that within five minutes after injection the dye is evenly distributed in the plasma of large peripheral blood vessels.

The difficulty of deciding the point lies in a correct interpretation of dye concentrations in a series of samples taken at brief intervals after an injection. Chart 1 poses the problem. The necessity of extrapolating the curve back to the time of injection makes it important to decide when mixing is complete. Gilder and her associates, convinced

that the peak of the hump represents even distribution of the dye, say that projection of latter portions of the curve may introduce errors as large as 10 to 20 per cent in plasma volume estimates.

It is our opinion that the interpretation of Gregersen, Gibson and their associates is the correct one,—that the hump of the curve (Chart 1) is due to the process of mixing, which takes place rapidly in the actively moving blood but more slowly in sluggish or stagnant portions

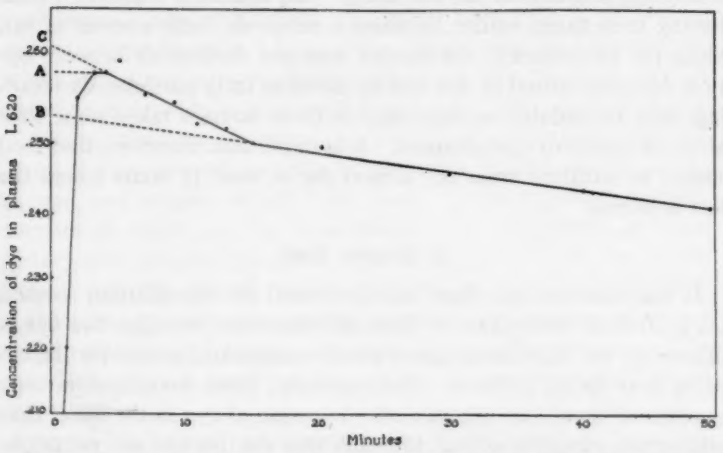

CHART 1. Concentration of T-1824 in plasma following a single injection. The dye was given intravenously at zero time. The problem is: When is mixing complete, and which portion of the curve, therefore, should be extrapolated to zero time to give the proper value to be used in calculating plasma volume? Early investigators used the value *A*; Gregersen uses the value *B*; Gilder *et al.* recommend the use of value *C*.

of the vascular system, and that the last part of the curve represents the disappearance rate. An analogous curve (minus the complicating factor of dye disappearance) can be produced readily in a flask of water connected by a tube to a second flask of water. A small amount of dye injected into the first flask diffuses rapidly through its water so that samples taken at short intervals show increasing concentrations of the dye. Later samples show decreasing concentrations, however, due to slow diffusion of the dye into the second flask. Eventually,

when mixing is complete, the curve flattens out. It is the projection of this flat portion of the curve which permits a correct calculation of the volume of water in the two flasks.

6. FATE OF T-1824 IN THE BODY

One of the advantages claimed for T-1824 is that it is eliminated from the blood stream more slowly than vital red and many other dyes used in plasma volume studies. Comparatively little has been written, however, about the mechanism of its disposal. Smith (17) reported that brilliant vital red injected into the blood passes gradually in large quantities into lymph and tissue fluids, that it is taken up by phagocytes until an equilibrium is reached between the dye in those cells and the dye in plasma, and that it is eliminated slowly by the liver. Similar observations were made with niagara blue. It has been assumed that T-1824 behaves similarly. Evans and Scott (18) found that large daily doses of T-1824 by mouth resulted after 13 days in the appearance of minute crystals of the dye in macrophages of the subcutaneous tissues of the abdomen, whereas vital red similarly given appeared in these cells in much larger quantities. Gregersen says that T-1824 in the circulation combines with plasma proteins (6), particularly with albumin (20). Gibson and Evans (10), who believe that the dye is removed from blood principally by phagocytosis in the reticulo-endothelial system, did not find dye in urine from normal kidneys, in transudates, or in cerebrospinal fluid. They assumed that the dye passes fairly promptly into the lymph, but did not attempt to find it in thoracic duct lymph. So far as we are aware, the presence of T-1824 in bile, though suspected (19), has not been demonstrated heretofore.

The following is a summary of our observations: (1) T-1824 injected intravenously passes slowly into the tissues of healthy dogs. Very large doses of the dye, which are not toxic (19), cause gradual visible staining of the skin, sclerae, and oral mucous membranes, as well as intestines, kidneys, gall bladder, and other internal structures. This staining of normal tissues is maximal after several hours. If deep, the color does not disappear completely for many days. Normally, the staining of urine, intestinal contents and serosal fluid is slight or absent.

(2) The escape of T-1824 from the blood vascular system may be abnormally rapid and pronounced in traumatized regions. In shocked dogs we have found no evidence of unusual loss of dye in the body tissues generally, however. A study of gross dye leakage as a measure of capillary permeability in different types of trauma and in shock will be published separately.

(3) T-1824 which escapes from the blood into normal or traumatized tissues is evidently taken up slowly by the lymphatics. Lymph glands become stained. Thoracic duct lymph collected with a cannula shows perceptible staining within 12 minutes of the time large amounts of dye are injected. In one experiment in which an untraumatized, anesthetized 13 kg. dog received 200 mgm. of dye intravenously in a single dose, maximal concentration of dye in the thoracic duct occurred about $2\frac{1}{4}$ hours after the injection; thereafter the concentration of dye in the lymph decreased slowly, maintaining a ratio of about 2:3 with the concentration of dye in circulating plasma. Similar observations were made with small doses of T-1824 except that the appearance of dye in the thoracic duct could not be detected for 30 minutes or more after injection. Under ordinary circumstances, of course, the dye in the lymphatic ducts is returned slowly to the blood at the base of the neck. This process, slow enough under ordinary circumstances, is retarded by general anesthesia, and almost ceases during shock.

(4) After very large intravenous injections of T-1824, its presence in the common bile duct can be demonstrated by soaking strips of filter paper in the bile for 15 minutes, then washing them in running water for an hour. Most or all of the bile pigment is removed by the water, leaving a waterfast blue stain on the paper. A rough idea of the concentration of dye in the bile can be obtained from the depth of the blue color on the paper. In one test in which a dog received 17.5 mgm. of T-1824 per kg. of body weight, dye first appeared in the bile 30 minutes after injection, reached maximal concentration during the second hour after injection, and subsequently showed gradual decrease in concentration as long as the animal was observed (four hours). Whether any of this excreted dye is absorbed from the intestinal lumen, we do not know.

(5) Chemically, T-1824 is very stable, as other investigators have

pointed out. We have found that the color of the dye is not altered appreciably by contact with plasma, whole blood, or minced muscle for 48 hours in vitro. It makes no difference whether this contact takes place in the presence of air, pure oxygen, pure nitrogen, or carbon dioxide.

We believe, therefore, that T-1824 injected into the blood of a healthy dog disappears because it diffuses slowly through capillary walls, is taken up by phagocytes, and is excreted in bile. Should a blood vessel be opened experimentally or accidentally, or should capillaries be sufficiently damaged, the dye escapes relatively rapidly along with the plasma proteins; but under ordinary circumstance it accumulates gradually in the intercellular fluid, whence it passes slowly into lymphatic channels and is returned, in part at least, to the blood. Presumably this slow circuit continues until all the dye has been eliminated from the body.

7. DISAPPEARANCE RATE

The rate of disappearance of T-1824 from the blood stream was estimated by Dawson, Evans and Whipple (2) to be five to eight per cent per hour. Gregersen (4) speaks of 5 to 10 per cent per hour. Gibson and Evans (10) report that in four normal persons the average rate of disappearance was six per cent during the first hour after injection but decreased to about two per cent 20 to 24 hours later.

The usual method of estimating the disappearance rate is to inject a quantity of dye intravenously, allow time for mixing, and then remove several samples of blood at intervals of 10 minutes or longer. Concentration of dye in the plasma (or serum) of these samples is determined, and the values obtained are plotted against time. The slope of a line drawn through these points is thought to indicate the rate of disappearance. Actually, however, this is not a curve of dye disappearance; it is only a curve of dye concentration in samples. These two curves are not necessarily identical. Failure to recognize that fact is one reason, we believe, for unsatisfactory results with the dye-dilution method.

The concentration of dye in blood depends upon two factors, the absolute amount of the solute and the volume of the solvent. Thus, given a constant plasma volume, loss of a portion of the dye present

would result in a reduced concentration of dye in the circulation. But, as far as dye concentration is concerned, a similar effect would be obtained in the absence of dye disappearance if the plasma was diluted by a shift of water from the tissues. Any combination of these two variables, dye removal and plasma dilution, may occur. The time-concentration curve of dye, therefore, is the algebraic sum of two unknown curves, namely, the curve of dye disappearance and the curve of plasma dilution. We have, so to speak, a single equation with two unknowns, and our problem is to find a way to resolve it. It should be noted in this connection that loss of whole blood or whole plasma from the circulation does not of itself alter the concentration of dye in the remaining blood because the amount of solute and volume of solvent are reduced in the same proportion.

Our investigations indicate that the disappearance of T-1824 is a function of the total mass of the dye in the circulation rather than of its concentration in plasma. Furthermore, the rate of disappearance seems to be constant in a given animal irrespective of the quantity of dye present. These two statements are not contradictory. A dog with a disappearance rate of five per cent per hour loses one-twentieth of his total intravascular dye in an hour whether the total quantity of dye present happens to be 2, 5, or 10 mgm. This rate is unaffected, therefore, by loss of dye, re-injection of dye, or changes in dye concentration due to intravenous infusions or to shifts of water into and out of the vascular system.

Shortly after dye is injected it begins to leave the blood stream because of diffusion, phagocytosis, and hepatic excretion. Theoretically, one would expect each of these activities to proceed at a logarithmic rate, as do so many physiologic processes where an imbalance is being adjusted. In that case the disappearance rate would also be logarithmic, since it is the algebraic sum of the three individual rates. It is difficult to say how much influence the relatively slow return of dye by lymph to the blood stream has upon the rate of dye disappearance. In three experiments we ligated the large lymph ducts at the base of the neck, and were unable to detect any significant alteration in the disappearance rate as a result, but Gregersen (20) in similar experiments found that there was some effect.

Our study of disappearance curves in the manner about to be de-

scribed has led us to conclude that T-1824 does in fact tend to leave the circulation at logarithmic rates; that is to say, if six per cent of the dye leaves the blood during the first hour, six per cent *of the remaining dye* leaves during the second hour, and so on. This logarithmic rate of dye removal appears to be constant for at least six or eight hours after every injection. Each dog seems to have a specific rate of disappearance, a rate which does not vary much from day to day or month to month. One of our dogs examined daily for more than a week, and subsequently at intervals for several months, showed a rate of 3.8 per cent per hour after every injection of dye. Two other control animals studied repeatedly always had disappearance rates of 7.5 and 6.1 per cent respectively. Individual rates in our entire group of animals ranged from 3.2 to 11.8 per cent per hour. It appears, furthermore, that the rate of disappearance is unaffected by nembutal or ether anesthesia, by variations in the amount of dye injected, or by many of the procedures which we have used to produce shock experimentally. Beginning six to eight or more hours after injection a slower rate of disappearance may be observed.

Gibson and Evelyn (9) state that when a second dye injection is given, the "effect of this residual dye is compensated for automatically by reading all the new dyed samples against a new center setting obtained by first setting the galvanometer to 100, with the dyed dye-free sample, exactly as though it contained no dye." Or, alternatively: "All the dyed samples including the dyed dye-free are read after setting to 100 with the original dye-free sample. The L value is obtained from the extrapolated G values in the usual way and is corrected for residual dye by subtracting from it the L value of the dyed dye-free sample." These procedures fail to take into account the fact that the residual dye continues to disappear after new dye is injected. The dyed dye-free sample cannot properly be considered a standard since there are progressively diminishing amounts of the residual dye in succeeding samples. When the procedures just described are followed, deceptively steep disappearance slopes are obtained after re-injection because the loss of both residual and new dye is attributed to loss of new dye alone.

We have avoided that difficulty by reading all of our samples against the original dye-free sample, a procedure which simply corrects for the

more or less constant natural coloring matter of plasma; consequently, our curves represent the disappearance rates of all intravascular dye, residual as well as new. To calculate the plasma volume from a reinjection of dye, we subtract the L value of the dyed dye-free sample from the extrapolated L value, and use this difference for the L value in Gibson's formula, as will be shown in the following section (formulas d and e).

The use of time-concentration slopes to measure and follow plasma volumes has been attended, therefore, by at least four sorts of errors: (1) Those which arise from failure to recognize and take into consideration fluid shifts into or out of the circulation during the test; (2) those due to projection of disappearance slopes with straight lines instead of logarithmic curves; (3) those which result from using dyed dye-free samples for standards; and (4), in the "indirect method" the error which may come from the unwarranted assumption that plasma volume does not change during a period of 12 to 16 hours prior to an experiment. In a single determination of plasma volume, extrapolation of the time-concentration curve to the time of dye injection is so short that, even if fluid shifts are ignored and the extrapolation is made with a straight line, the error in the final calculation is not usually large; but a long forward extension of the time-concentration slope is liable to many of the errors just described, and resultant calculations of plasma volume may be far from accurate.

8. A METHOD OF DETERMINING THE DISAPPEARANCE RATE

The method which we have employed to measure the factor of fluid shifts and thus deduce the true disappearance rate will be best described by giving an example.

A dog weighing 20.22 kg. was given three widely spaced injections of T-1824. Immediately before each injection a sample of blood was taken, and after each lot of dye presumably had become evenly distributed through the circulation, additional samples were taken at relatively short intervals, as indicated in Chart 2. Between the second and third injections of dye the animal was bled 443 cc. of blood in order to produce a mild degree of "shock." This bleeding represented a loss of 269 cc. of plasma. L values of the samples are plotted against time on a logarithmic scale. Lines AB, DE, and GH are time-concentra-

tion curves, extrapolated, which differ from those used by previous workers in that these are logarithmic curves, and in that they represent total dye concentration; that is, residual as well as re-injected dye.

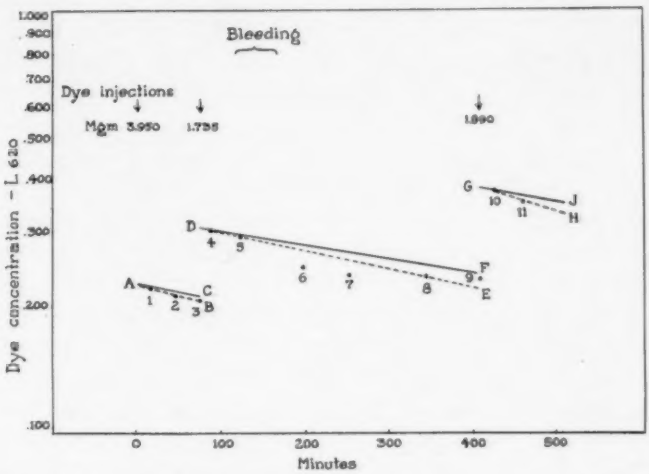

CHART 2. A study of dye concentrations in the plasma of a dog subjected to a fairly large hemorrhage. Broken lines represent time-concentration curves, solid lines the true disappearance rate.

Tentative estimates of plasma volume at the time of the three injections are made as follows: (see section 4, formula (a)):

(c) $$P.V. = \frac{\text{mgm. dye} \times K}{L_{620}\,\text{extrap.}} = \frac{3.950 \times 66.74}{.224} = 1176\ \text{cc.}$$

(d) $$P.V. = \frac{\text{mgm. dye} \times K}{L_{620}\,\text{extrap.} - L_{620}\,\text{ddf}} = \frac{1.735 \times 60.74}{.306 - .208} = 1180\ \text{cc.}$$

(e) $$P.V. = \frac{\text{mgm. dye} \times K}{L_{620}\,\text{extrap.} - L_{620}\,\text{ddf}} = \frac{1.990 \times 66.74}{.381 - .232} = 890\ \text{cc.}$$

At the time of the second injection of dye, three conditions are known: (1) The concentration of dye in sample 3 ($L_{ddf} = .208$); (2) the expected plasma volume; that is, the original plasma volume less the volume of plasma removed by sampling ($PV_{exp} = 1176 - 9 = 1167$ cc.); and (3) the measured plasma volume; that is, the volume

estimated independently from the second injection of dye (PV_{meas} = 1180). A fourth unknown condition is the concentration of dye which would be shown by sample 3 had there been no shift of water into or out of the circulation between the times of the first and second injections of dye, in which case disappearance by ordinary physiologic processes would be the only factor concerned. This unknown concentration of dye (L_x) can be determined, however, because of the relationship

(f) $$\frac{L_x}{L_{diff}} = \frac{PV_{meas}}{PV_{exp}}$$

or, transposing,

(g) $$L_x = \frac{L_{diff} \times PV_{meas}}{PV_{exp}}$$

A moment's thought will show why the above relationship is true. If there had been no fluid shift at all, the L value of the dyed dye-free sample would equal the theoretical L value resulting from the single factor of dye disappearance, and the expected and measured plasma volumes would be the same. If, however, a quantity of water from the tissues had been added to the blood, concentration of dye in the dyed dye-free sample would be reduced in inverse proportion to the increase in plasma volume as shown by the re-injection.

In our example, therefore,

(h) $$L_x = \frac{.208 \times 1180}{1167} = .211$$

This procedure cancels out the factor of fluid shifts; consequently a line drawn from the extrapolated value to point .211 (curve AC in chart 2) represents approximately the true rate of dye disappearance. We must say "approximately" because there remains a possibility that an irregular shift of fluid occurred between the time of the initial injection of dye and the taking of the first sample, in which case point A would be wrongly placed. A fluid shift of that sort might be suspected if a significant change in plasma protein concentration occurred between samples 0 and 1, or for other reasons to be discussed later.

Having established the curve AC as the approximate disappearance rate, we then draw lines DF and GJ parallel to it on the assumption that the disappearance rate remains constant for at least six or eight

hours after each dye injection, and that this is true even after the circulation has been depressed by a large hemorrhge.

9. PLASMA VOLUME CHANGES

In our example, the three injections of dye permit three independent, fairly reliable estimates of plasma volume, one at the time of each injection. For practical reasons the number of such injections that may be given during the course of an experiment is limited. The value of the method is greatly enhanced, therefore, by the proper use of individual samples of plasma, to measure the variations in plasma volume which occur between injections of dye.

Deviations of L values of samples from the curve of disappearance do not indicate changes in plasma volume, as some investigators have supposed; they show, rather, that dye in the circulation has been diluted or concentrated by net gains or net losses of water by plasma. Obviously, loss of blood or whole plasma does not of itself alter the concentration of dye in the remaining plasma, although the absolute quantity of dye in the circulation is reduced thereby; conversely, shifts of water into or out of the vascular system change the concentration of dye, but do not alter the absolute quantity of dye in the blood or the rate of its disappearance. Thus it is possible for plasma volume to be greatly reduced without any change of dye concentration in samples; on the other hand, a hemorrhage combined with a compensatory fluid shift equal in size to the amount of plasma lost would cause pronounced dilution of the dye in samples even though the plasma volume had not changed. It is clear then that simply to correct the initial plasma volume by the deviation of samples, as recommended by Gibson and his associates (9, 10), does not provide reliable information regarding changes in plasma volume; it is necessary to take into account also the plasma removed or otherwise lost during the experiment and the effects of transfers of water upon the disappearance curve.

We have devised a method of following plasma volume changes which includes all the factors just mentioned. The plasma volume at the time any sample is taken is calculated by the formula

$$(i) \qquad PV_s = PV_{ap} \times \frac{L_{de}}{L_s},$$

where PV_s is the plasma volume to be determined; PV_{exp} is the expected plasma volume; that is, the volume calculated from the preceding dye injection (or from the preceding sample) less the volume of plasma lost between the time of the injection (or the time of the preceding sample) and the taking of the present sample; L_{dc} is the L value of the properly adjusted disappearance curve at the time of the present sample; and L_s is the L value of the sample. The use of this formula is indicated in the following example.

In the experiment already described and illustrated in Chart 2, the plasma volume at the time of the first injection of dye was estimated to be 1176 cc. (see section 8, equation (c)). When the first sample was taken, the expected plasma volume would also be 1176 cc. since no blood had been removed in the interim. The L value of this sample ($L_s = .220$) is a little less, however, than the L value of the disappearance curve ($L_{dc} = .221$). Consequently, the plasma volume at the time of the first sample would be

(j) $$PV_1 = 1176 \times \frac{.221}{.220} = 1181 \text{ cc.}$$

That is to say, an estimated 5 cc. of water from the body tissues was added to the plasma during the mixing period. Now in drawing the first sample of blood, three cubic centimeters of plasma was removed from the circulation; the expected plasma volume at the time of the second sample, therefore, would be $1181 - 3 = 1178$ cc. The L value of the second sample must now be compared not with the line AC (Chart 2) but with a curve parallel to AC passing through point 1, since, if there has been no further fluid shift, it is that quantity of dye in that concentration which is disappearing at the established rate. The plasma volume at the time of the second sample, therefore, is

(k) $$PV_2 = (1181 - 3) = \frac{.215}{.214} = 1183 \text{ cc.}$$

The second sample of blood happened to be a larger one containing 7 cc. of plasma. The volume at the time of the third sample, then, would be

(l) $$PV_3 = (1183 - 7) \times \frac{.209}{.208} = 1181 \text{ cc.}$$

This amount, less the quantity removed in taking sample 3, gives a volume of 1178 cc. At this point dye was re-injected and the independent determination shows a plasma volume of 1180 cc. (see section 8, equation (d)). This close agreement between calculated and measured volumes is expected, however, since the curve AC was so drawn as to bring about that very result (formula (f)).

This step-by-step calculation is now continued throughout the experiment, as shown in Chart 3. It should be noted that the calculated

CHART 3

Step-by-Step Calculation of Plasma Volume Changes from Individual Samples

DYE INJECTED	SAMPLE	MEASURED PV	EXPECTED PV	L_{dc}/L_s	CALCULATED PV	PLASMA REMOVED
mgm.		cc.	cc.		cc.	cc.
	0			000		
3.950		1176		.221/.220		
	1		1176	.221/.220	1181	3 in sample
	2		1178	.215/.214	1183	7 in sample
	3		1176	.209/.208	1181	3 in sample
1.735		*1180*	1178		*1178*	
	4		1180	.301/.301	1180	3 in sample
	5		1177	.294/.290	1192	6 in sample
						269 in bleeding
	6		917	.273/.248	1009	4 in sample
	7		1005	.236/.241	986	4 in sample
	8		982	.224/.238	923	5 in sample
	9		918	.226/.232	891	3 in sample
1.990		*890*	888		*888*	
	10		890	.375/.374	893	3 in sample
	11		890	.365/.361	900	

volume at the time of sample 9 closely approximates the independent measurement of plasma volume by the third injection of dye. This agreement is not forced. It is taken as evidence that the tentative extrapolation values and choice of disappearance rate were correct. If, in this experiment, additional dye injections had been given, and if these had shown similar agreements between calculated and measured plasma volumes, we should feel even more certain that the true disappearance rate had been found and that it had remained constant.

Calculations of plasma volume do not always work out smoothly as in the example just given; that is to say, after calculations like those

illustrated in Chart 3 have been carried out, discrepancies may be found between calculated and measured plasma volumes at the time of the third and subsequent injections of dye. Such discrepancies might conceivably be due (1) to technical errors, (2) to an inconstant disappearance rate, or (3) to significant fluid shifts occurring between the time of dye injection and the removal of the ensuing sample.

It has been our custom to consider discrepancies greater than a few cubic centimeters as beyond the range of probable technical error.

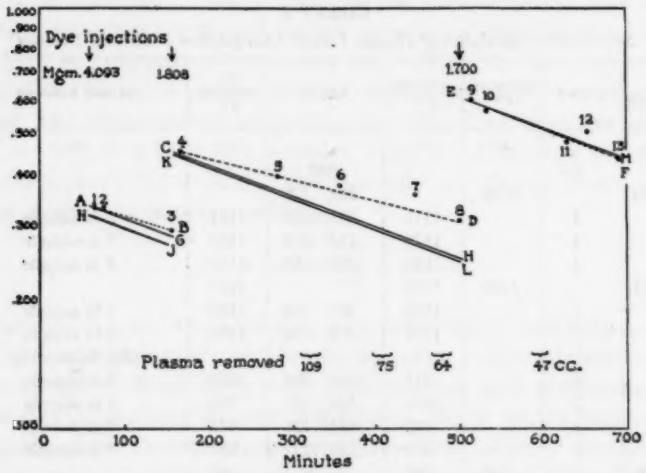

CHART 4. Dye concentrations and disappearance curves in the plasma of a dog subjected to acute plasmapheresis. The animal died shortly after sample 13 was taken.

Variations in the disappearance rate within six to eight hours of dye injection do occur in dogs but most likely only under certain exceptional conditions to be discussed shortly. We believe that these differences between calculated and measured plasma volumes are usually indicative of irregular fluid shifts which occur during the mixing periods, since, by postulating such fluid shifts and adjusting the curves accordingly, the differences can be made to disappear.

The following experiment shows how these adjustments are made. A dog weighing 20.34 kg. was subjected to acute plasma removal (plasmapheresis) as indicated in Chart 4. The time-concentration

curves *AB*, *CD*, and *EF* clearly do not represent the disappearance rate since calculations based on them (the procedure illustrated in Chart 3 being used) show large disagreements between calculated and measured plasma volumes.

	PV CALC.	*PV* MEAS.	DIFFERENCE
	cc.	*cc.*	*cc.*
At the first injection		803	
At the second injection	798	749	49
At the third injection	457	378	79

By employing the method described in section 8 which takes fluid shifts into account, an approximately correct disappearance rate is determined. This is represented by parallel curves *AG*, *CH*, and *EM*. Calculations based on these curves show much better agreement.

	PV CALC.	*PV* MEAS.	DIFFERENCE
	cc.	*cc.*	*cc.*
At the first injection		803	
At the second injection	749	749	0
At the third injection	364	378	14

This amount of disagreement cannot be lessened by the choice of any other disappearance rate, but it can be lessened by assuming that a fluid shift occurred between the time of the first injection of dye and the taking of sample 1, as indicated by curves *HJ*, *KL*, and *EM*. Calculations based on these curves show almost perfect agreement.

	PV CALC.	*PV* MEAS.	DIFFERENCE
	cc.	*cc.*	*cc.*
At the first injection		828	
At the second injection	766	763	3
At the third injection	379	378	1

The size and the direction of this postulated shift can be determined mathematically by a statistical method, which might be termed "distribution of error," but they are more simply ascertained by eye-fitting of curves with trial-and-error calculation of volumes. This procedure, it should be said, is not a random juggling of figures; it is, rather, a

search for the single satisfactory answer to the problem. That is to say, in this experiment no other disappearance rate and no other initial fluid shift than the ones assumed will result in as close an agreement between calculated and measured volumes. We conclude, therefore, that these assumptions are valid.

It should be clear from the foregoing discussion that at least three properly spaced injections of dye are required to determine the disappearance rate and to follow satisfactorily the variations in plasma volume which occur in an acute experiment. The accuracy and certainty of the results are greatly increased by each additional injection of dye. Another example will be given in order to illustrate this important point. In this experiment a trained, healthy, unanesthetized dog was given five injections of dye at 6-hour intervals and samples of blood were removed hourly. Altogether 84 cc. of plasma was removed from the circulation. Food was withheld but the animal was permitted to drink water freely. The disappearance rate of dye was found to be 6.1 per cent per hour. Irregular transient fluid shifts, some of them fairly large, are indicated by deviations of the points from the curves of disappearance in Chart 5. Plasma volumes at the times of the injections are as follows:

	PV CALC.	*PV* MEAS.	DIFFERENCE
	cc.	*cc.*	*cc.*
At the first injection		784	
At the second injection	798	796	2
At the third injection	742	741	1
At the fourth injection	667	667	0
At the fifth injection	652	654	2

Such close agreement between calculated and measured volumes cannot be obtained in this experiment by assuming any other disappearance rate than 6.1 per cent per hour, or by starting with any other value for the original plasma volume than 784 cc. These figures are, therefore, believed to be correct.

We have found two conditions which appear to reduce the rate of dye disappearance; namely, a severe grade of anoxic anoxia and extreme, prolonged hypotension (mean pressure less than 40 mm. Hg)

due to continuous intravenous injection of histamine. Under those conditions the dye leaves the circulation much more slowly than usual, and in some instances the process of disappearance seems to be temporarily suspended, as shown by subsequent re-injections and measurements of the absolute amounts of residual dye in the blood. In these experiments it is not possible to follow the variations in plasma volume

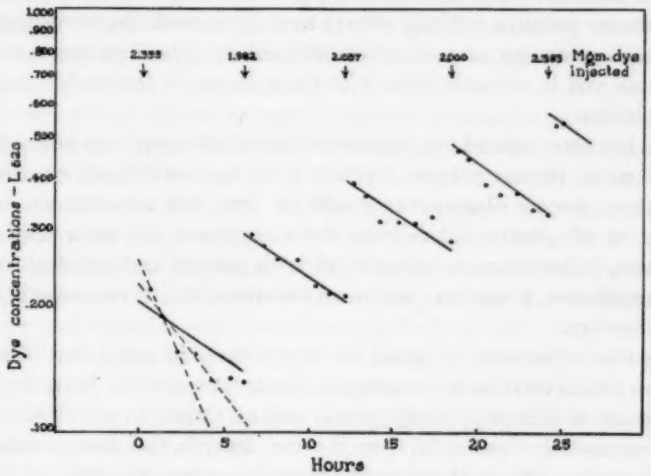

CHART 5. Dye concentrations in the plasma of a healthy, unanesthetized dog during a 25-hour period. The points represent dye concentrations of individual samples, and the solid-line curves the disappearance rate. Obviously, in this experiment, a single dye injection and use of the ensuing time-concentration curve (one of the broken lines) for the dsappearance rate would have led the investigators astray.

as accurately as in Chart 3, since there is an indeterminable time-lag in the reduction, and later in the restoration, of the usual disappearance rate.

10. MEASUREMENT OF PLASMA LOSS IN TRAUMATIC SHOCK

Experimental traumatic shock is commonly produced by pounding the muscles of a leg, burning a portion of the body surface, exposing and handling the intestines, injecting minced muscle into the peritoneal

cavity, and like procedures, which cause severe local injury to tissues. Fluid, which in many cases resembles serum, collects in or oozes from these injured tissues, and there is concomitant reduction in plasma volume. It has been generally assumed, therefore, that this fluid comes from the blood, leaking from damaged capillaries and ruptured blood vessels. Some writers go further, claiming that in late stages of shock capillaries throughout the body become abnormally permeable to plasma proteins. Many efforts have been made by investigators to estimate the amounts of excess fluid collected in these traumatized regions, and to compare them with the amounts of plasma lost by the circulation.

As has been pointed out, the use of individual samples to follow fluctuations in plasma volume depends upon two conditions; namely, a constant, known disappearance rate for dye, and accurate measurement of all plasma taken from the circulation. If, as a result of trauma, indeterminable amounts of both plasma and dye leak from the capillaries, it may be questioned whether either of these conditions can be met.

In this connection it should be kept clearly in mind that T-1824 leaves the circulation in two ways. First, there are the physiological activities of diffusion, phagocytosis, and excretion, in which the dye is progressively "extracted," as it were, leaving the plasma volume unchanged. This is the process referred to when we speak of "dye disappearance." It is probable that under normal conditions T-1824 diffuses through endothelium bound to protein molecules, and at the rate that those proteins ordinarily diffuse from blood into the intercellular fluid; but at the same time undyed proteins are being added to the plasma at about the same rate, so that concentration of proteins in plasma remains constant although the concentration of dye decreases. The second way in which T-1824 leaves the circulation is along with the abnormal escape of plasma, a process which reduces the absolute quantity of dye but not its concentration in the circulation. These two methods of dye removal, the one physiologic, the other pathologic, may occur simultaneously, but they appear to be independent of each other. We have already presented evidence (section 8 and 9) to show that the rate of disappearance of residual dye is unaltered by experi-

mental bleeding and plasmapheresis. In like manner large internal hemorrhages remove quantities of dye from the circulation but apparently do not modify the usual rate of disappearance. Presumably, a severe trauma may likewise permit escape of whole blood or plasma (and dye) externally or into the tissues without altering the rate of removal of residual dye by physiological processes. If so, it is permissible to use pre-established disappearance curves to study changes in plasma volume following trauma, provided the amounts of whole plasma escaping through the damaged blood vessels and capillaries can be determined.

The volume of plasma escaping from the circulation as a result of trauma may be estimated by an adaptation of the mathematical procedure described in section 9. The following example will show how these calculations are made. Incidentally, this experiment also illustrates an interesting new approach to the problem of traumatic shock.

A dog weighing 21.76 kg., under nembutal anesthesia, had the muscles of a hind leg pounded with a mallet, several hundred strokes being administered. Care was taken not to break the bones, lacerate the skin, or injure the large vessels. The animal gradually developed signs of shock and died 19 hours after the trauma. No therapy was given. The leg slowly increased in size; at autopsy it was found to contain a good deal of fluid, but relatively little extravasated blood. Bisection with Blalock's technique showed that the traumatized leg weighed 220 gms. more than the opposite leg.

Dye studies are shown in Chart 6. The first two dye injections establish the disappearance rate, *AB* and *CD*. The deviation of point 5 is due to an unaccountable sudden shift of fluid from the tissues to the circulation, a shift reflected in a synchronous lowering of plasma protein concentration and hematocrit. The curve *CD* may be projected to *E*, the time of the third dye injection, but its relation to points 6, 7, 8, and 9 cannot properly be used to calculate changes in plasma volume unless the amounts of plasma lost from the circulation during this period are known.

At the outset the dog had an estimated plasma volume of 1,088 cc., and at the time of the second injection of dye, 1,082 cc. When the third injection was given he had only 939 cc. The amount of whole

Some experiments are not written here.

plasma lost from the circulation between the time of the second and the time of the third injections may be estimated by means of the formula.

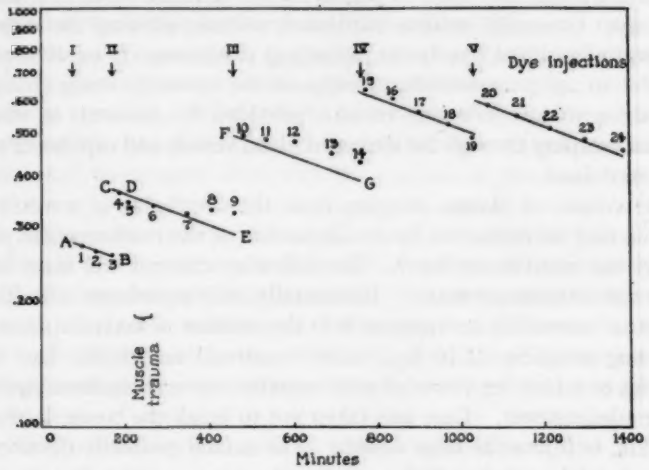

CHART 6. Dye concentrations in the plasma of a dog subjected to severe muscle trauma followed by shock and death. No therapy was given.

(m) $$(PV_{II} - x) \times \frac{L_{de}}{L_s} = PV_{III},$$

where PV_{II} is the plasma volume at the time of the second injection of dye
x is the amount of plasma lost,
L_s is the L value of sample 9,
L_{de} is the synchronous L value of the disappearance curve (point E), and
PV_{III} is the plasma volume determined independently by the third injection.

Substituting our values in the formula, we have

(n) $$(1082 - x) \times \frac{.289}{.328} = 939$$

(o) $$x = 17 \text{ cc.}$$

According to this calculation, then, only 17 cc. of plasma was lost in the period between the second and third injections of dye, a quantity which corresponds almost exactly with the volume of plasma in samples 4 to 9; and the reduction in plasma volume following trauma must be

explained otherwise than by escape of whole plasma from the vascular system.

In the same way an estimate can be made of plasma loss between the third and fourth injections. The curve FG represents the disappearance rate. Plasma at the time of the third injection was 939 cc., and at the fourth injection 814 cc. Applying formula (m).

(p) $$(939 - x) \times \frac{.387}{.434} = 814$$

(q) $$x = 26 \text{ cc.}$$

Again, this calculated loss, 26 cc., approximates the known loss in samples, 22 cc.

A similar calculation shows that 13 cc. of plasma was lost during the period between the fourth and fifth injections, and that, as it happens, is exactly the amount of plasma removed in sampling.

These results seem to indicate that in this particular experiment no whole plasma escaped from the circulation other than the amounts removed in samples, and that the decrease in plasma volume which occurred after the trauma was due rather to loss of undyed fluid (water) from the plasma.

These conclusions, as well as the foregoing calculations, are based upon the assumption that the disappearance rate of dye remains normal and constant following muscle trauma. We feel that that assumption is justified not only for reasons already advanced but also because an estimate of plasma volume changes in this experiment, in the manner illustrated in Chart 3, shows almost perfect agreement between measured and calculated volumes at the time of each re-injection of dye. Such close agreement cannot be obtained by the assumption of any other disappearance rate.

It is not within the scope of this paper to discuss the sources of the fluid which collected in the traumatized leg; but it may be noted in passing that the gain in weight of the leg (220 gms.) plus the weight of the plasma removed in samples (77 gms.) nearly equals the calculated reduction in circulating plasma volume (316 gms.).

11. FLUID SHIFTS

Since water is continually entering and leaving the circulation, a constant plasma volume depends to a great extent upon a balance

between the inflow and outflow of such fluid. Minor disturbances of this dynamic equilibrium occur, even under virtually normal conditions, as Chart 5 clearly shows; and under certain abnormal circumstances, large fluid shifts (net gains or losses of water) may take place.

The size of these shifts of water can be estimated by comparing two sets of values: (a) The plasma volumes calculated in the manner already described; and (b) the volumes obtained when the initial

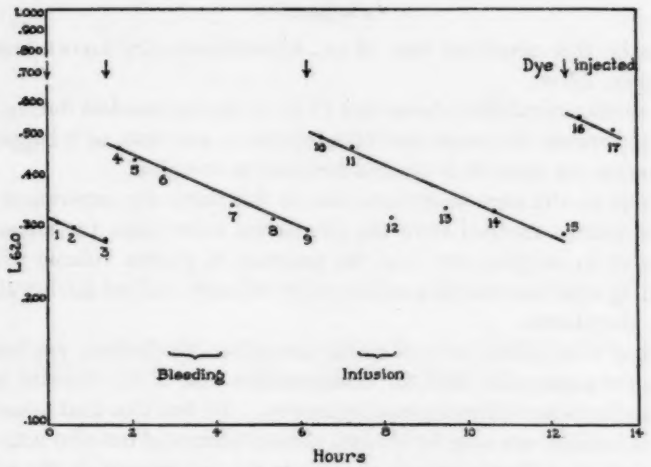

CHART 7. Dye concentrations and disappearance curves in the plasma of a dog subjected to severe bleeding and treated subsequently with an infusion of physiological salt solution.

plasma volume, determined by the first dye injection, is simply corrected for the amounts of plasma removed or otherwise lost and the amounts of water injected intravenously. One more experiment will be reported to indicate the method of making these calculations.

A dog weighing 11.3 kg. was bled 300 cc., a loss of 143 cc. of plasma, whereupon signs of shock appeared. Some time later, when the slowly falling mean blood pressure had reached 40 mm. Hg, 300 cc. of physiologic salt solution was given intravenously. Temporary improvement in the animal's condition was noted; the pressure rose to

about 70 mm., was maintained at that level for an hour, and then began to fall again. Death occurred 5½ hours after the infusion.

Chart 7 shows graphically the effects of the bleeding and the infusion upon the concentration of dye in the plasma, and Chart 8 interprets

CHART 8

Calculation of Plasma Volumes and Fluid Shifts in a Dog Subjected to a Large Hemorrhage and Treated Unsuccessfully by a Saline Infusion

SAMPLE	MEASURED *PV*	CALCULATED *PV*	PLASMA REMOVED	WATER INJECTED	UNCORRECTED *PV*	NET GAIN OR LOSS OF WATER BY PLASMA
	cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
	576				576	
1		576			576	0
			3			
2		577			573	+4
			3			
3		578			570	+8
			8			
	570	*571*		3		
			0			
4		570			565	+6
			3			
5		581			562	+19
			8			
6		566			554	+12
			146			
7		440			408	+32*
			7			
8		427			401	+26
			3			
9		417			398	+25
			3			
	417	*414*				
			0	2		
10		415			397	+18
			3			
11		410			394	+16
			3	300		
12		547			691	−144†
			5			
13		452			686	−234
			4			
14		403			682	−279
			8			
15		378			674	−296
			4			
	373	*374*				
			0	2		
16		371			672	−301
			4			
17		364			668	−304

Measured plasma volumes are determined by dye injections, calculated plasma volumes by the method illustrated in Chart 3; uncorrected volumes are the original plasma volume corrected for amounts of plasma removed and amounts of water (including dye solution) injected; and the difference between "calculated" and "uncorrected" volumes measures the gain or loss of water by plasma.

* Dog bled.

† Infusion.

these effects in terms of plasma volume and fluid shifts, and incidently, shows what happened to the infused salt solution. It will be seen that the plasma volume was fairly constant during the control period of the experiment (samples 0 to 6); that the bleeding reduced the plasma

volume about as much as would be expected, that is to say, relatively small amounts of water were transferred from the tissues to the blood in compensation for the hemorrhage (samples 7 to 11); that the infusion, instead of increasing the plasma volume by 300 cc., as might be expected, raised it by less than half that amount, the rest having run rapidly out of the vascular system; even this residual water was soon lost also, so that the plasma volume at the time of sample 14 was lower than it had been before the infusion was administered.

COMMENT

The volumetric determinations given in the foregoing sections are not so precise as the specific figures might imply. In the first place, they are based upon assumptions which, though supported by a certain amount of experimental evidence, cannot be considered as proved,—that the mixing of dye and blood is complete within 15 minutes in normal narcotized dogs, or within 20 to 30 minutes in shocked animals; that thereafter the dye remains evenly distributed in the intravascular plasma; that T-1824 does not change color in vivo; that extrapolation of the latter part of the dye-concentration curve, as recommended by Gregersen, is the correct procedure; that dye disappears from the circulation by physiologic processes at logarithmic rates which are characteristic for each animal, which are constant for at least six to eight hours after each injection of dye, and which are unaffected by bleeding, plasmapheresis, or most of the methods used to produce shock experimentally; that unexpected changes in dye concentration (deviations of L values from the disappearance curves) are proportional to and are, therefore, a measure of fluid shifts into or out of the plasma.

Furthermore, since the dye-dilution method consists of many consecutive procedures each of which is subject to errors, the effects of these errors upon the final result are cumulative. If there are five steps, for example, and each step has an error of ± 1 per cent, the error in the result will be $\sqrt{.01^2 + .01^2 + .01^2 + .01^2 + .01^2} = \pm 2.24$ per cent. Thus, it is important that the error in each step be reduced to a minimum.

The same sort of effect is observed in our stepwise calculations of plasma volume changes and fluid shifts (Charts 3 and 8). The L values of the samples, which we have used as though they were exact,

are in fact each liable to technical error of at least ±1 per cent, to which must be added the errors involved in drawing and reading the charts.[3] Although these errors are balanced, they are additive when calculations are based on a series of samples. Under the circumstances, however, the best that can be done is to treat the *L* readings as though they were accurate. Calculations based upon that assumption probably bring us as near as we can come to a correct answer.

We do not believe that the dye-dilution test in its present form can be considered a precise method of measuring or of following plasma volume. We do feel, however, that it probably is the best method available for these purposes, that it is capable of yielding information which is approximately correct, and that it is an exceedingly valuable tool in the investigation of such conditions as experimental shock.

SUMMARY

The dye-dilution method of measuring plasma volume is believed by the authors to be sound in principle, but its use is beset with many inherent and potential errors. A number of technical modifications are suggested which are designed to increase the precision of various steps in the procedure.

The success of the method depends largely upon an accurate determination of the disappearance rate of dye and the proper interpretation of deviations of dye concentration from the established curve of dye disappearance.

It is important to distinguish between the time-concentration curve of dye in plasma and the curve of dye disappearance. The former is a compound curve, the algebraic sum of dye disappearance and diluting effects of fluid shifts. A method of determining the true disappearance rate is given.

By dye "disappearance" is meant the removal of dye from the vascular system by physiological processes such as diffusion, phagocytosis, and excretion. Although the rate of disappearance differs widely among dogs, each animal appears to have a characteristic rate which is unaffected by the quantity of dye injected or (with certain

[3] To minimize these errors we have used large sheets of semi-logarithmic graph paper measuring 25 x 60 cm., on which the curves can be drawn and read with considerable accuracy.

exceptions) the condition of the animal. For at least six or eight hours after each injection dye disappears from the blood at a constant logarithmic rate. Experimental removal of blood or plasma, or acute blood or plasma loss from disease or trauma, have no direct effect upon the concentration of dye in the vascular system because in such cases dye and plasma have been removed in the same proportion. Furthermore, these losses of blood or plasma do not appear to alter the rate of physiological disappearance of residual or re-injected dye.

By repeated injections of dye and frequent sampling of the plasma, variations in plasma volume and shifts of water into and out of the vascular system can be followed closely throughout an experiment. It is also possible by this means to estimate the quantity of plasma lost in traumatized areas or in the body tissues generally, and to observe the fate of intravenous infusions. Methods of carrying out these tests and of making the calculations are given in detail.

The revised method has proved reliable and extremely useful in our study of experimental shock.

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THE INFLUENCE OF DIETARY FAT UPON THE EXCRETION OF UROBILIN[1,2]

HUGH W. JOSEPHS, L. EMMETT HOLT, JR., HERBERT C. TIDWELL AND CHARLOTTE KAJDI

From the Harriet Lane Home of the Johns Hopkins Hospital and the Department of Pediatrics, Johns Hopkins University

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Although the hemolytic properties of soaps *in vitro* had long been known, it was only in the early part of the present century that attention was called to lipid substances as a possible cause for hemolytic anemias. Faust and Tallquist (1) in 1907 extracted a hemolytic substance (believed to be cholesterol oleate) from the fish tape worm, and reported that this would produce a hemolytic anemia in experimental animals. The view that bothriocephalus anemia was due to the absorption of hemolytic lipids met with general acceptance, and it was not long before other anemias were attributed to the ingestion of fats. Czerny advanced the view that the nutritional anemia seen in infants fed for a prolonged period on cow's milk was due to an injurious property of the milk fat, and his pupil Glanzmann (2) obtained evidence in support of this idea. Glanzmann used the excretion of urobilin in the stools as a measure of hemolysis; he found an increased urobilin output in cases of nutritional anemia, and reported that this was further increased by a diet high in milk fat, whereas elimination of the diet fat decreased it to the merest traces. It was an easy step to attribute the newly discovered goat's milk anemia (described by Scheltema (3) in 1916) to the fat of goat's milk, which is peculiar in its relatively high content of caproic, caprylic and capric acids.

In recent years, however, the view that fats are responsible for these anemias has met with serious setbacks. The fact that bothriocephalus anemia responds to liver therapy (4) casts doubt upon the accepted

[1] The expenses of this investigation were defrayed in part by the John Howland Memorial Fund and in part by a grant from Mead Johnson and Company, Evansville, Indiana.

[2] A preliminary report of this work was published in the Proceedings of the American Society for Clinical Investigation. *J. Clin. Invest.*, **17**: 532, 1938.

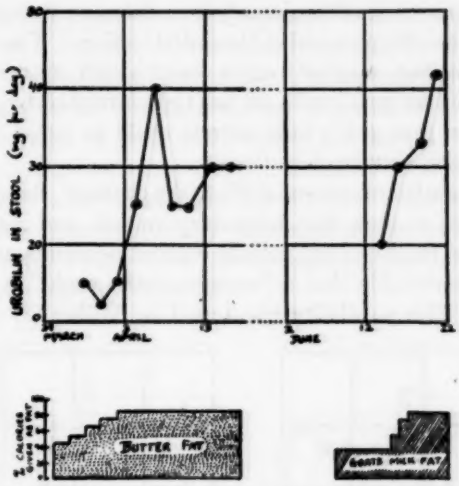

FIG. 1. PATIENT O. W.

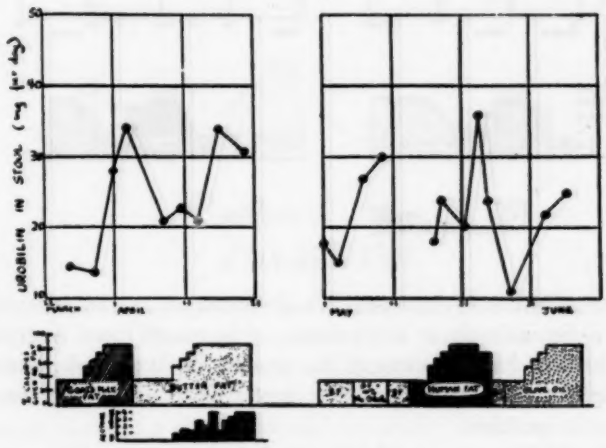

FIG. 2. PATIENT J. S.

view of Faust and Tallquist. It is now abundantly clear that the nutritional anemia of infancy is due to a dietary deficiency rather than

to a toxic factor; it results primarily from defective hemoglobin formation rather than from excessive blood destruction. The same appears to be true of that uncertain entity—goat's milk anemia. Complete vindication of the goat's milk fat has been furnished by Baar (5) who demonstrated that goat's milk anemia could be cured merely by increasing the daily intake of goat's milk.

In spite of earlier observations (6) to the contrary, there is now abundant evidence to show that laboratory animals and man can be fed high fat diets for prolonged periods without becoming anemic. It is, of course, conceivable that a "compensated anemia" is produced by such diets. This possibility was tested by Müller (7) who failed to

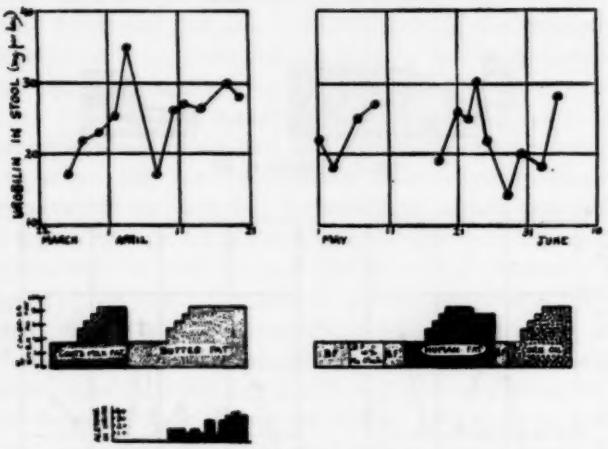

FIG. 3. PATIENT W. S.

find any evidence of increased blood formation in the reticulocyte counts of her animals, or any evidence of increased blood destruction in the form of hemosiderosis of the organs. It is possible, however, that the method used by Müller to detect blood destruction was not sufficiently sensitive.

Total urobilin output has been regarded as a very sensitive measure of blood destruction, but the studies of the influence of dietary fat upon this function are few and contradictory. Glanzmann (2) made pertinent observations on one child only. His results were not con-

firmed by Adler and Sachs (8), although they were supported by those of Salomon (9). Because of the conflicting data we undertook to reinvestigate this question with more accurate methods of urobilin estimation than were available to the original investigators. We were also interested to see if differences in the behavior of particular fats could be detected by this method, inasmuch as some of us were already engaged in comparative studies of fats in infant nutrition (11). That such differences might exist was suggested by Glanzmann, who noted in one subject that a diet high in sesame oil gave higher urobilin values than one high in butter fat.

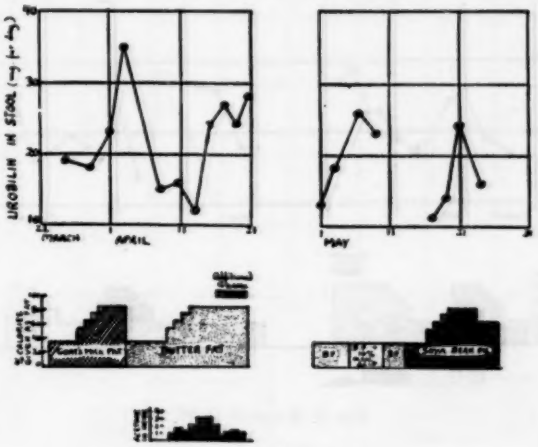

FIG. 4. PATIENT J. H.

Our studies were made upon six normal infants between 5 and 8 months of age. These infants were given diets of skimmed milk in which various fats had been incorporated by homogenization. The fats studied were butter fat, goat's milk fat, human body fat, corn oil, olive oil, soya bean oil and an odd carbon fat consisting of a mixture of the triglycerides of pentadecylic and margaric acid. Observations were also made with diets to which free fatty acids and soaps had been added. The quantity of fat ingested was varied, between 35 and 85 per cent of the calories being supplied in this form.

During the periods of study the infants were kept upon metabolism frames to insure complete collections of the excreta. Urobilin determinations were made by the method of Elman and McMaster (10). Separate urine determinations were made on some patients, but since it was found that significant variations in the urine urobilin did not occur,[3] these determinations were not continued. The stools were collected and analyzed daily, but because of the diurnal variation in urobilin output the results reported are averaged for two or three days at a time. The accompanying charts (figures 1 to 6) show the effects

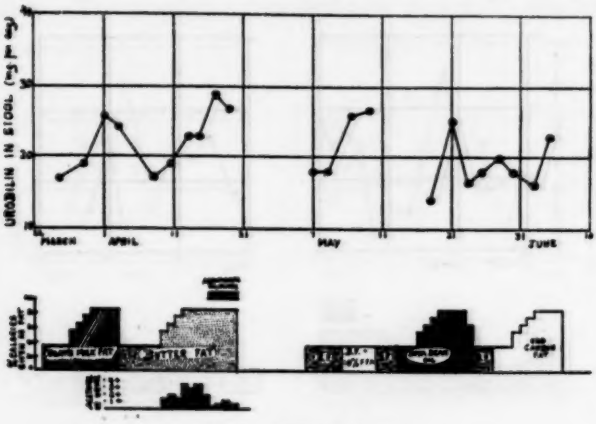

FIG. 5. PATIENT G. H.

of qualitative and quantitative variations in the fat of the diet upon the excretion of urobilin in the stools.

It will be seen from the appended charts that our results confirm Glanzmann's original observation that an increase in the fat of the diet causes a rise in the output of urobilin in the stools. All the animal

[3] In the normal individual the urine urobilin constitutes only 5 per cent or less of the total urobilin excretion. The fluctuation of the urobilin excreted in the stools is many times greater than the total urinary excretion. It is therefore obvious that when total urobilin excretion is being considered, as in the present study, the urinary excretion may be disregarded. Under other circumstances, as for instance in tests of hepatic function, the ratio of urinary excretion to total excretion is important.

and vegetable fats studied seemed to produce about an equal increase in urobilin output; when the fat was increased from 35 to 85 per cent of the caloric intake the urobilin excretion rose from 15 or 20 milligrams a day to 30 or 40 milligrams. Such an increase is, of course, small as contrasted with that seen in post-infectious states where noticeable anemia develops; under such circumstances the urobilin output may rise to 60 or 100 milligrams per day, and in the hemolytic anemias it may be well in excess of this. The response is none the less a very constant one. It is also clear that the rise in urobilin excretion is a significant one. On the basis of a large number of obser-

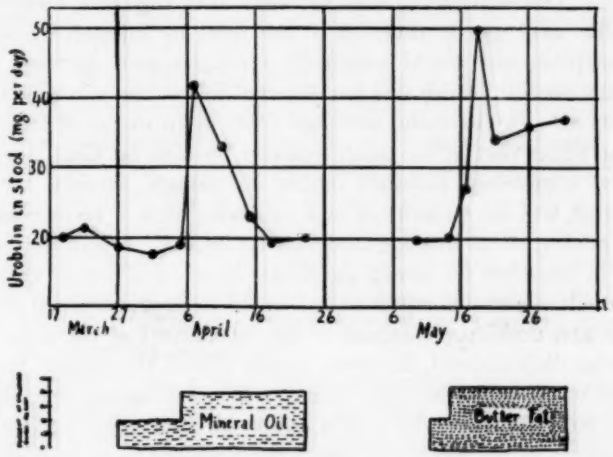

FIG. 6. PATIENT R. S.

vations made by one of us in infants (14) it has been established that under the conditions of the present study the variation in fecal urobilin output is ±20 per cent. An inspection of charts shown here indicates the constancy of the urobilin output in the control period, which in patient R. S. (Figure 6) was of twenty days' duration.

DISCUSSION

The finding of an increased urobilin output in the stools on high fat diets raises a number of questions as to the interpretation of this phenomenon. Must it be attributed to an increased blood destruction

or are there other possible explanations? And how is the effect brought about?

Although there is no longer any question in regard to the fact that increased blood destruction leads to an increased fecal urobilin output and that the latter is probably the best method available for following blood destruction, we cannot overlook the fact that other processes may influence urobilin output. Any factor causing *interference with the reabsorption of bile pigment from the intestine* might result in an increased fecal urobilin output and it is conceivable that the presence of increased fat in the intestine on a high fat diet might bring about this result. This explanation does not seem to fit the facts, however. If urobilin were merely diverted to the feces by interference with its reabsorption one would anticipate a simultaneous decrease in the urinary urobilin, which was not observed in two cases in which it was measured. Furthermore, although there is an excess of fat present in the upper part of the small intestine on high fat diets, this fat is almost completely absorbed during its passage through the small intestine, and the quantity of fecal fat shows little or no increase from that present on an average moderate fat intake. If solubility of urobilin in intestinal fat were a significant factor in diverting urobilin to the stools one would expect to find in the feces proportional increases in fat and urobilin. Analyses of the fat content of the feces on the high fat diets showed, however, figures within the normal limits for babies on usual levels of fat intake.[4] It would appear then that the sharp increase in urobilin output cannot be attributed to a rise in the fat content of the stools. It seems more reasonable to attribute it to an excess of bile pigment brought to the intestine. In order further to test the possibility that solution of urobilin in the fecal fat was not causing diversion of the latter to the feces, an experiment was performed on a normal diet to which 5 grams of mineral oil was added. This diet yielded a fecal output containing roughly twice as much oily material capable of dissolving urobilin as any of the companion experiments reported herewith, and if the effect under discussion had been due to diversion of dissolved urobilin one would have anticipated a sustained increase in urobilin output as long as the mineral oil supple-

[4] Excluding the single experiment on odd carbon fat, the fecal fat on the high fat diets ranged between 1.65 and 4.02 grams per day (average 2.73 grams).

ment was continued. The results actually obtained are shown in Figure 6. Although the addition of mineral oil produced a definite increase in urobilin output this was not sustained as it was in the case of the high fat diet, the urobilin excretion returning promptly to its original level. The explanation of the temporary increase in pigment output on the mineral oil is considered below.

A second possibility that must be excluded before concluding that the increased urobilin output is due to increased blood destruction is that the high fat diet has merely caused a *temporary "washing out" of the stores of bile pigment.* It is well known that fats cause an evacuation of the gall bladder. Such an effect should not, however, be a sustained one. In order to test out this possibility we continued the period of high fat in two of our patients (O. W. and R. S.) for more than two weeks. It will be noted (Figures 1 and 6) that the initial high urobilin output was not sustained, but that a level was nevertheless maintained well beyond that observed on the usual fat intake. We are inclined to believe that the initial rise in urobilin output is due in part to an increased flow of bile—in other words, to washing out of stores of pigment—but that the less striking, sustained urobilin increase during subsequent days cannot be so explained. It seems not unlikely that the urobilin rise in the mineral oil experiment was due to some temporary effect of washing out body stores of pigment, since this effect was altogether temporary.

The failure to obtain a rise in reticulocytes upon high fat diets does not in our opinion militate against the view that increased blood destruction was occurring, for it is quite possible that the degree of increased red cell destruction was not sufficient to cause a reticulocyte increase, although sufficient to be detected by the more sensitive method of urobilin excretion.[5]

It occurred to us that we might obtain confirmatory evidence of blood destruction by studying the iron metabolism. An increase in blood destruction should free iron as well as bile pigment, and this might be followed by an increased excretion of iron.

[5] From a priori considerations a rise in reticulocytes would not have been expected under these circumstances. In our experience it has been observed only in the presence of sufficient blood destruction to cause a 50 to 100 per cent rise in urobilin excretion and an appreciable degree of anemia.

The accompanying table shows the iron balance on subjects J. S. W. S., J. H. and G. H. during the period of rising and high butter fat diet. It is very clear that during the first part of the high fat period there is a marked increase in the output of iron, causing a negative balance in three out of four cases. This increase was not sustained, however, for during the second four days of the high fat diet the iron retention was essentially normal.

Had the increased iron output been sustained one might feel reasonably sure of the presence of increased blood destruction, but the data actually obtained give only equivocal information on this point. It is possible that the temporary iron loss represents washing out of reser-

TABLE 1

Effect of Increasing Fat Intake on Iron Balance

		J. S.	W. S.	J. H.	G. H.
Period 1. Low fat and increasing fat, 4/9–12/33	Intake (mgs. per day)	0.91	0.87	1.03	1.00
	Output (mgs. per day)	0.71	0.70	0.72	0.85
	Balance (mgs. per day)	+0.20	+0.17	+0.31	+0.15
Period 2. High fat 4/12–15/33	Intake (mgs. per day)	0.59	0.58	0.68	0.65
	Output (mgs. per day)	0.86	0.53	1.03	1.36
	Balance (mgs. per day)	−0.27	+0.05	−0.35	−0.71
Period 3. High fat 4/15–19/33	Intake (mgs. per day)	0.54	0.52		0.68
	Output (mgs. per day)	0.53	0.43		0.47
	Balance (mgs. per day)	+0.01	+0.09		+0.21

voirs; it is known for instance that a shift to a high fat diet is accompanied by loss of water and of other minerals, and it may be that the increased excretion of iron at this time is an analogous process. On the other hand, it is equally possible that iron is actually liberated in increased quantities, but that after a few days a new balance is obtained, reabsorption of iron keeping pace with increased secretion in the bile. Our iron experiments therefore neither support nor disprove the presence of increased blood destruction.

By a process of exclusion our experiments have led us to the conclusion that the increased urobilin output in the stools on a high fat diet must be attributed largely to increased blood destruction. This conclusion, expressed in our preliminary report has since been strength-

ened by experiments recently reported by Freeman and his collaborators (13) who have obtained direct evidence of a hemolytic principle in thoracic duct lymph and in lipemic serum after a high fat meal.

The mechanism of this phenomenon remains to be explained. Is it due to the fat itself, to some particular lipid component or to some metabolic change produced by the high fat diet? We have carried out some studies to throw light on this point. One of the first possibilities that occurred to us was that ketone bodies might be responsible, for as a rule the increase in urobilin occurred only after the dietary fat had been increased to a ketogenic level. This possibility we attempted to rule out by adding glucose at the height of the ketosis and urobilin rise to see if a reduction in ketosis would be accompanied by a corresponding fall in urobilin output. It will be seen from the charts that in the two patients given extra glucose (patients J. H. and G. H.) the urobilin output remained as high as in the controls (patients J. S. and W. S.) although the acetone in the urine fell promptly. It would therefore appear that ketosis is not responsible for the increase in urobilin output.

A further possibility that occurred to us was that increased hemolysis on a high fat diet might be due to the absorption of increased quantities of free fatty acids or soaps. This was suggested by the known hemolytic action of soaps. Traces of free fatty acids and of soaps are said to pass the intestinal barrier and it seemed likely that the quantities might be greater on a high fat diet. We have not attempted post absorptive measurements of free fatty acid or soap in the blood, but we have studied the effect on urobilin output of adding free fatty acids and soaps to the feeding. To our astonishment it was found that a relatively small addition of either free fatty acid or soap to the diet (e.g. 10 per cent of the caloric intake) produced a rise in fecal urobilin output comparable to that brought about by a large increase in the dietary fat. These results are shown in the graphs of patients W. S., J. H. and G. H. Until direct measurements are made on the blood in the post-absorptive state, we cannot reach a definite conclusion on this point, but the evidence seems to us at least suggestive that the hemolytic effects of high fat diets are due to increased absorption of soap.

The question still remains as to whether this phenomenon has any practical significance. It would seem that in the normal subject, a process which leads neither to anemia nor to reticulocyte increase is hardly to be feared. On the other hand it is conceivable that in abnormal subjects whose bone marrow is already subjected to strain, a high fat diet might add unnecessarily to the burden. This is a question that only further observations will determine.

SUMMARY

1. Normal infants fed on high fat diets exhibit an increased output of urobilin in the feces. The effect is independent of the type of fat fed.

2. Free fatty acids and soaps, even in comparatively small quantities, cause a similar rise in urobilin output.

3. Various possible explanations of these findings are discussed, the most satisfactory being that absorption of increased amounts of soap, on a high fat diet or a diet to which free fatty acid or soap have been added, leads to increased hemolysis.

4. A high fat diet causes a sudden but temporary increase in the output of iron in the feces. Possible explanations for this phenomenon are discussed.

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A DEHYDRATING FIXATIVE FOR GENERAL USE, INCLUDING A DESCRIPTION OF TECHNIQUES AND STAINS FOR PARAFFIN AND CELLOIDIN SECTIONS

WILLIAM B. VANDEGRIFT

From the Department of Pathology, School of Medicine, The Johns Hopkins University, Baltimore

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Satisfactory sections for critical microscopic study of human and animal tissues are obtained in 48 hours by the use of the fixative described below.

1. Ethyl alcohol 95% (or isopropyl alcohol 99%)	80.0 cc.
2. Formalin (full strength)	12.0 cc.
3. Glacial acetic acid	4.5 cc.
4. Picric acid (dried before weighing)	4.0 gm.
5. Mercuric chloride	0.2 gm.
6. Urea	0.5 gm.

The alcohol, formalin and glacial acetic acid are mixed together in this order to form a mixture in which the picric acid, mercuric chloride and urea are dissolved separately and in this order. Solution of each is made rapidly and is completed before the next is added.

GENERAL PROPERTIES AND USES OF THE FIXATIVE

The fixative corrects the faults and, at the same time, utilizes the special qualities of fixation by the alcohols. Therefore it is suitable for general use. Furthermore, the simultaneous fixation and dehydration of tissues render it useful in the rapid methods of preparing tissue sections. Stained sections prepared in 48 hours are only slightly inferior to sections prepared over a longer period of time. For these reasons, an outstanding value of the fixative is its use in surgical pathology where a final diagnosis within 2 days after biopsy or operation is highly desirable.

The volatile nature of the fixative and the ease with which it stains objects a deep yellow, limit its value as a fixative for autopsy material if a large number of blocks are fixed in an open pan.

Refixing of tissue which has been fixed in formalin and Kaiserling's

solutions for long and short periods of time considerably improves the original fixation. The use of the fixative for autopsy material which has been fixed previously for 3 to 7 days in 10 per cent formalin is recommended. The refixing of formalin fixed animal tissues improves the cutting as well as the primary fixation.

Preparation of Tissue Sections

Blocks of tissues are cut no more than 5 mm. thick. They are fixed 16 to 48 hours. Fixation is done preferably over a layer of cotton which is covered with filter paper. In this manner each side of the block is fixed equally and the flat surface of the filter paper minimizes the necessity of trimming the blocks after fixation. Blocks of tissue acquire a permanent shape in the fixative within 10 minutes; they are firm enough to trim in 2 hours. Tissues adhere firmly to the uncovered bottom of the bottle unless they are added after the fixative has been put in. The bottle should be agitated occasionally during the next 10 minutes. Centrifuged sediment of fluid from body cavities is loosened from the tube several minutes after the fixative has been added. Blocks are placed directly in the dehydrating agent following fixation; they are not washed.

The dehydrating agents are placed in pint Mason jars containing a layer of anhydrous copper sulphate 0.5 cm. thick. This is overlaid with cotton which, in turn, is covered with filter paper. Similarly, cotton and filter paper are placed in the Mason jars containing the intermediate and clearing agents. The dehydrating agent in the first jar is changed when the copper sulphate in the second jar first shows blue or first becomes hydrated. The intermediate and clearing agents are changed when they become discolored darkly by the picric acid. A minimum of three changes of the dehydrating agent is made.

Paraffin solvents are nearly saturated with paraffin and they are used at room temperature. Paraffin and paraffin preparations having melting points of 56 to 58°C. are preferred; melting points of 50 to 52°C. are satisfactory. Paraffin impregnation requires a minimum of 3 hours after acetone and chloroform and 6 hours after benzol; three changes of paraffin are preferred. Sectioning of imbedded blocks of tissue entails no change in the established methods used for tissues fixed with other fixatives. Paraffin sections attached to the slides by Mayer's albumin-glycerin mixture adhere firmly after heating to 45°C. for 2 hours.

Procedures

The first procedure listed below has been used in the surgical pathology laboratory for 2½ years. Skin, muscle, bones, colloid goitres, uterine fibromata and other tissues difficult to impregnate with paraffin are prepared more satisfactorily by the second procedure. Blocks of formalin- and Kaiserling-fixed tissues are placed directly in the fixative and treated exactly like the blocks of fresh tissue.

Procedure I: 2 days.

Fixation: 16 hours to 24 hours.

Acetone 2½ hours: 3 changes; ½ hour for the first change, 1 hour for the second and third changes.
Paraffin 3 hours: 3 changes of 1 hour.
Imbed and cut in the afternoon.
Stain the next morning.

Procedure II: 2 days.
Fixation: 16 hours to 24 hours.
Acetone 3 hours: 3 changes of 1 hour.
Paraffin 20 hours: 3 changes: 2 hours for the first and second changes; overnight in the third change.
Imbed and cut in the morning.
Stain in the afternoon.

Small pieces of tissue, such as curettings, may be treated according to Procedure III, which is a 24 hour method.

Procedure III: 1 day.
Fixation: Overnight.
Acetone 2 hours: 3 changes; ½ hour in the first and second changes, 1 hour in the third change.
Paraffin 1½ hours: 3 changes of ½ hour each.
Imbed and cut in early afternoon.
Stain in the late afternoon.

In general, the slides prepared by the rapid methods are suitable for special studies. Minute structures are demonstrated, however, more clearly by the following 4 procedures which disregard the advantages of obtaining sections rapidly.

Procedure IV: 4 days.
Fixation: 2 days.
Ethyl alcohol, absolute, or 99 per cent, 8 hours: 3 changes; 2 hours in the first change, 3 hours in the second and third changes.
Benzol-paraffin 8 per cent at room temperature overnight.
Paraffin 6 hours: 3 changes of 2 hours.
Imbed.
Cut in the morning.
Stain in the afternoon.

Procedure V: 6 days.
Fixation: 2 days.
Ethyl alcohol, absolute, or 99 per cent isopropyl alcohol 8 hours: 3 changes: 2 hours in the first change, 3 hours in the second and third changes.
Aniline 23 hours: 2 changes.
Benzol-paraffin 8 per cent at room temperature overnight.
Paraffin 24 hours: 3 changes; 4 hours in the first and second changes; 16 hours in the third change.
Imbed and cut in the morning.
Stain in the afternoon.

Procedure VI: Double imbedding. 11 days.

Fixation: 2 days.

Ethyl alcohol, absolute, or 99 per cent isopropyl alcohol 8 hours; 3 changes; 2 hours in the first change, 3 hours in the second and third changes.

Acetone-celloidin 4 per cent at room temperature 5 days.

Acetone 1 hour: 2 changes of ½ hour each (A.M. seventh day).

Aniline 24 hours: 2 changes.

Benzol 1 hour: 2 changes of ½ hour each.

Benzol- paraffin 8 per cent at room temperature 24 hours: 2 changes.

Paraffin 24 hours: 3 changes: 4 hours in the first and second changes; 16 hours in the third change.

Imbed and cut.

Stain the next day.

Procedure VII: Celloidin.

Fixation: 2 days.

Ethyl alcohol, absolute, 8 hours: 3 changes; 2 hours in the first change, 3 hours in the second and third changes.

Ether-ethyl alcohol, absolute, equal parts, overnight.

Celloidin in ether-ethyl alcohol absolute, equal parts: proceed according to the accustomed method.

Decalcification: Spicules of bones, such as are found in the nasal conchae and in the marrow of the femur, are adequately decalcified during fixation. In the following procedure no blocks are washed in water at any time. Fifty cc. of fluid are used for each change for each block which is suspended in the fluid. The 5 per cent nitric acid mixture is only moderately faster than the 1 per cent mixture, which is more desirable owing to the better preservation of the tissues during decalcification. Formalin is added to all fluids between fixing and refixing in order to maintain the preservation and proper consistency of the tissues. Refixing after decalcification restores the tissues and dissolves the crystals deposited in the tissues during decalcification. The cortex of the bone is placed in a position away from the edge of the microtome knife in order to reduce vibration during sectioning.

Fixation: 16 hours to 48 hours.

Blocks are decalcified in one of the two fluids until the test for calcium salts is negative. The fluid is changed twice daily.

Fluid (1) 1 per cent nitric acid in 10 per cent formalin

Fluid (2) 5 per cent nitric acid in 10 per cent formalin

Place blocks in a 3 per cent solution of sodium sulphate in 10 per cent formalin for 1 day.

Refix 1 day.

Proceed according to one of the above procedures.

Test for decalcification. Place 5 cc. of a 4 per cent solution of ammonium oxalate and 5 cc. of a 10 per cent aqueous mixture of "stronger ammonia water" (27–29 per cent NH_3) in a test tube. Pipette 1 cc. of the decalcifying fluid from the bottom and add to the test tube. The absence of the formation of a precipitate

in 5 minutes indicates adequate decalcification. A control test for the calcium salts normally found in tap water is carried out by taking 1 cc. saved from the last change of the decalcifying fluid before it is used. Decalcifying fluid made with distilled water and fluid containing small amounts of extracted fixative need no control testing.

Frozen Sectioning: A special fixative has been devised for frozen sectioning in order to obtain satisfactory paraffin sections of the refixed frozen blocks.

Water	58.5 cc.
Ethyl alcohol (95 per cent)	25.0 cc.
Formalin	10.0 cc.
Glacial acetic acid	5.0 cc.
Urea	1.5 gm.
	100.0 cc. or gm.

Blocks of tissue are removed from the fixative about 5 seconds after it has been quickly brought to a boil. They are rinsed in water, frozen and sectioned. Any of the usual stains, including fat stains, are applicable.

Stains

Following the removal of the paraffin from the sections in the usual way (xylol, absolute alcohol, 95 per cent alcohol, 50 per cent alcohol, water) they are then placed into a 1 per cent alcoholic solution of iodine (iodine crystals 1 gram, 95 per cent alcohol, 100 cc.) until they have become light brown (about 20 seconds). They are then rinsed in 95 per cent alcohol and bleached. The bleach consists of a 1 per cent mixture of "stronger ammonia water" in 95 per cent alcohol. The slides are immersed in the bleach for 3 minutes, washed well and stained. Bleaching allows more delicate and accurate staining by removing the picric acid retained in the tissues. Sodium hyposulphite, potassium metabisulphite and sodium chlorite are unsatisfactory bleaches. For reticulum and neuroglial stains a bleach containing 2 per cent of "stronger ammonia water" is used for 5 minutes. Long immersion of the slides in the iodine-alcohol solution distorts the staining affinities of the tissues. The use of a bleach containing an excessive amount of extracted iodine results in defective stains; hematoxylin stains are cloudy. Owing to the small number of mercurial crystals, the treatment with iodine may be omitted.

Hematoxylin and Eosin-Phloxine Stain: Harris' hematoxylin stain is preferred owing to its excellence and rapidity of staining. Phloxine is added to the eosin in order to obtain more brilliant staining of muscle and minute structures. The formula for the eosin-phloxine stain is:

Eosin Y	0.8 gm.
Phloxine	0.2 gm.
Alcohol 70 per cent	100.0 cc.

The sections are stained in Harris' hematoxylin for 5 minutes, differentiated briefly in acid alcohol (0.5 per cent hydrochloric acid in 95 per cent ethyl alcohol),

washed well and blued in ammonia alcohol (0.5 per cent "stronger ammonia water" in 95 per cent ethyl alcohol). The slides are washed well and placed in the eosin-phloxine stain for 1 minute. Following this, they are rinsed in 95 per cent ethyl alcohol and differentiated in absolute ethyl alcohol until the phloxine shades are accentuated.

Bacterial Stain: The aniline-crystal violet stains give superior staining of the Gram positive bacteria. Basic fuchsin is satisfactory for counter-staining owing to the deep staining of the Gram negative bacteria. The formulas for these stains are:

1. Crystal (or gentian) violet	2.0	gm.
Isopropyl alcohol 99 per cent (or 95 per cent ethyl alcohol)	20.0	cc.
Distilled water	78.0	cc.
Before using add 0.1 cc. of aniline to 2.0 cc. of stain and shake well.		
2. Basic fuchsin	0.05	gm.
Ethyl alcohol 95 per cent	5.0	cc.
Dissolve the basic fuchsin in the alcohol and add:		
Distilled water	95.0	cc.

The following Gram stain is a modification of the Brown and Brenn (1) Gram stain. Stain in the aniline-gentian violet for 3 to 5 minutes, wash and place in strong Lugol's solution for 1 minute; wash. Differentiate in a mixture of 1 part ether and 3 parts acetone until no more dye is extracted and wash in running water before the slide dries. Stain in the basic fuchsin for 3 to 5 minutes, wash, blot almost dry, rinse in acetone and differentiate briefly in a 0.1 per cent solution of picric acid in acetone. The sections are then rinsed briefly in acetone and run through 3 changes of xylol. Differentiation in the picric acid-acetone solution is the most critical step: nuclei and Gram negative bacteria should be red and cytoplasm should be yellow. Pre-staining by hematoxylin is omitted because a stain sufficient to resist replacement of the nuclear stain by the basic fuchsin also resists replacement of the hematoxylin stain of the Gram negative bacteria.

Acid Fast Stain: The aniline-basic fuchsin stains are preferred to the carbolic acid-basic fuchsin stains. The formula for an effective aniline-basic fuchsin stain is:

Basic fuchsin	2.0	gm.
Isopropyl alcohol 99 per cent (or 95 per cent ethyl alcohol)	20.0	cc.
Distilled water	78.0	cc.

Before using add 0.1 cc. of aniline to 2.0 cc. of stain and shake well.

The most satisfactory carbolic acid-basic fuchsin stain is Kinyoun's stain (dissolve 4 gm. of basic fuchsin in a solution of 8 gm. of crystalline carbolic acid in 20 cc. of 95 per cent ethyl alcohol; add distilled water while shaking to make up 100 cc.).

Gently steaming for 5 to 10 minutes is the most effective method of staining. Decolorize the stain briefly, or until the stain is deep pink, in 0.1 per cent hydro-

chloric acid in 95 per cent ethyl alcohol. Wash well in water and stain briefly (several dips) in a 1 per cent solution of methylene blue which contains 0.1 per cent of "stronger ammonia water." Wash well in water and differentiate briefly in the alcohols. Overstaining by the methylene blue entails prolonged differentiation in the alcohols which slowly decolorizes the acid-fast bacilli.

Neuroglial Stain: Following bleaching for 5 minutes in 2 per cent "stronger ammonia water" the slides are placed in a freshly made 0.25 per cent solution of potassium permanganate for 10 minutes. Wash and place the slides in a 1 per cent solution of oxalic acid for 5 minutes. Wash and stain in Mallory's phosphotungstic acid-hematoxylin stain for 1 to 3 days; differentiate rapidly in the alcohols.

Other Stains: The Masson trichrome stain employs a slightly heavier stain with Harris' hematoxylin than is usual. The sections are stained by the Ponceau dexylidine-acid fuchsin stain for 3 minutes, rinsed, differentiated in a 1 per cent solution of phosphomolybdic acid for 3 minutes. Without rinsing, stain in a 1 per cent solution of Light Green to which is added 1 per cent of glacial acetic acid, for 3 to 5 minutes. The stains are then fixed, after rinsing, for 3 minutes in a 2 per cent aqueous mixture of glacial acetic acid. Wash the slides in alcohol free of contamination by other stains. Over-staining in the Ponceau dexylidine-acid-fuchsin stain decreases the hematoxylin stain by replacing it. Excessive differentiation in the phosphomolybdic acid tends to render the connective tissues fast for the red dyes.

Granules of the hypophysis are stained by the trichrome stain. A 1 per cent solution of methyl blue, without added glacial acetic acid, is used for the basophile granules instead of the Light Green stain. Acidophile chromophobes stain brown, whereas basophile chromophobes stain light blue. Basophiles, which stain deep blue, and basophile chromophobes are not absolutely differentiated.

Wright and Giemsa stains are best employed by diluting in alkalinized distilled water and staining overnight. Add 2 cc. of Wright stain or 1 cc. of Giemsa stain to 100 cc. distilled water containing 0.5 cc. of a 0.5 per cent solution of sodium bicarbonate. Employ other stains according to the described methods.

DISCUSSION

Methyl alcohol, ethyl alcohol, isopropyl alcohol, acetone and diethylene oxide (dioxan) are dehydrating agents used commonly in histological techniques which require the removal of water from fixed tissues preparatory to imbedding in water-immiscible media. In addition, they possess the properties of fixing and hardening the tissues. The remarkable ability of these chemicals to fix, harden and dehydrate the tissues simultaneously presents obvious advantages. Unfortunately, however, they have faults of fixation which preclude their general use as tissue fixatives. The faults are incomplete fixation, the condensation of the periphery of the fixed tissue into a brittle shell

which hinders further penetration by the fixative, the lysing of hemoglobin, and the solution of fats and myelin. On the other hand, they are useful fixatives in a specialized sense. Fixation of minute structures and bacteria is excellent.

Many efforts have been made to correct the faults of the fixation by the dehydrating agents by adding other fixing agents. The most notable early efforts were made by Carnoy (2, 3), in 1886, and by Mann (4), in 1893. Carnoy's fixative consisted of absolute ethyl alcohol 60 cc., chloroform 30 cc., and glacial acetic acid 10 cc. Mann's fixative consisted of absolute ethyl alcohol 100 cc., picric acid 4 gm., mercuric chloride 15 gm., and tannic acid 6 to 8 gm. Following the introduction of formalin as a general fixative by Blum (5), in 1893, mixtures of the dehydrating agents and formalin, and such mixtures containing other fixing agents, were tried but none proved as satisfactory for general use as Blum's 10 per cent aqueous mixture of formalin. Furthermore, the formalin precipitated the hemoglobin, lysed by the dehydrating agents in the tissues, as dark brown, opaque, insoluble crystals. Ethyl alcohol was the dehydrating agent most often used with formalin because it excelled the others in fixation and dehydration. The usual formula for this elaborated dehydrating fixative has been formalin 10 cc. and alcohol 90 cc.; occasionally glacial acetic acid has been added in amounts varying from 5 to 10 per cent.

The most notable fixatives containing a dehydrating agent, formalin and other fixing agents were those described by Ohlmacher (6) in 1897, Bing and Ellerman (7) in 1909, Duboscq-Brasil (8), in 1905, and Van Leeuwen (9), in 1908. Ohlmacher's fixative consisted of absolute ethyl alcohol 80 cc., chloroform 15 cc., glacial acetic acid 5 cc., and mercuric chloride to saturation (about 20 gm.). It was devised for the fixation of the brain and the spinal cord and he recommended it also for "ordinary" tissues. Bing and Ellerman's fixative consisted of acetone 9 parts and formalin 1 part; it was used for the fixation of medullated nerves. Duboscq-Brasil's fixative consisted of picric acid 1 gm., glacial acetic acid 15 cc., formalin 60 cc., and 80 per cent alcohol 150 cc. They used their fixative for the preservation of special structures, such as the mitotic figures in the sporulation of the Urospora. Van Leeuwen's fixative consisted of a 1 per cent solution of picric acid in absolute ethyl alcohol 6 parts, chloroform 1 part, formalin 1

part, and, before using, glacial acetic acid ½ part. It was recommended for the preservation of insect larvae. The fixatives of Mann (4) and Carnoy (2, 3) were likewise devised for special uses. None of these fixatives, however, corrected the faults of the dehydrating agents. They were unsatisfactory, therefore, for general use.

Consequently, the use of the dehydrating fixatives has been limited to the preservation of particular structures and to fixation in rapid paraffin techniques, which sacrificed adequate fixation for the gain in time resulting from simultaneous fixation and dehydration of tissues. The following discussion relates to the dehydrating fixative described in this paper.

The Fixative: Any change in the order and manner in which the ingredients are added diminishes the effectiveness of the fixative. Dissolving the last three ingredients simultaneously causes the formation of a precipitate on standing. The solution of the solid ingredients is associated with the formation of a slight precipitate which is removed by filtering through a hard or thick filter paper.

Picric acid and glacial acetic acid react with the alcohol and formalin to form ethyl formate and ethyl acetate. Ethyl formate is formed in sufficient amount to mask the odor of formalin after 24 hours, whereas the formation of ethyl acetate is considerably slower; the character of the fixation is unaltered. The fixative permeates and destroys cork and rubber and reacts with iron and aluminum. It is inflammable to a degree comparable with that of the ethyl alcohol. There is no evidence that it is explosive under conditions of normal use.

The Fixing Properties of the Ingredients: The following outline lists the fixing properties of the ingredients in a morphological sense. The ingredients have complementary and supplementary actions, the sum of which characterizes the fixation. Cells, connective tissues, minute tissue structures, and bacteria are adequately fixed. The minute structures include elastic fibres, reticulum, axones, glia, Nissl substance, cilia, inclusion bodies, pigments, granules, mucus, glycogen, keratin and others.

1. Ethyl alcohol: excellent minute structures and bacteria; fair nuclei and cytoplasm; poor collagen and muscle.

2. Formalin: good collagen and muscle; fair nuclei and cytoplasm; poor minute structures.

3. Glacial acetic acid: excellent nuclei with enlargement and improvement of the delineation of chromatin; fair collagen and muscle; poor cytoplasm.

4. Picric acid: general effect with emphasis on nuclei, cytoplasm and minute structures.

5. Mercuric chloride: general effect with emphasis on minute structures and nuclei.

6. Urea: general effect with emphasis on collagen, muscle and cytoplasm.

Absolute ethyl alcohol is superior to the 95 per cent ethyl alcohol but the slight improvement does not warrant the added expense. Isopropyl alcohol has similar, but slightly inferior fixing properties to those of the ethyl alcohol. Methyl alcohol and acetone in place of the ethyl and the isopropyl alcohols are inferior. The formation of a gas and a precipitate occurs with the use of dioxan.

Picric acid is the most important single ingredient. It prevents the formation of the brittle shell and the overhardening effect of the alcohol and prevents the precipitation of the lysed hemoglobin by the formalin.

Several amides have been tried in place of the urea (carbamide); benzamide (1 per cent) approaches the effectiveness of urea. Tannic acid (0.3 per cent) and chromic acid (0.1 per cent) may be used with or in place of the mercuric chloride. Tannic acid accentuates basement and cellular membranes and collagen, whereas chromic acid accentuates collagen. Each is a poor nuclear fixative. Chromic acid diminishes the amount of the reticulum by gathering it with the collagen. Tannic acid and chromic acid are compatible in solution in the fixative but their respective fixing reactions are incompatible. Chromic acid is dissolved without exerting pressure on the crystals in order to avoid a flash of flame in the fixative, thereby causing loss of the chromic acid by the oxidation of the alcohol. Tannic acid is added to the fixative before use owing to the formation of a precipitate on standing.

Fixation: The fixative maintains a delicate balance between ingredients which harden and shrink and soften and swell the tissues, and also between those which increase and decrease the rate of penetration of the tissues by the fixative. The ingredients causing hardening and shrinkage are alcohol and mercuric chloride; softening and swelling are caused by glacial acetic acid, picric acid, formalin and

urea. A slight increase of the formalin, glacial acetic acid, picric acid, urea and mercuric chloride results in excessive hardening of the tissues; a slight decrease results in inadequate fixation. Glacial acetic acid is the most critical ingredient in this regard; a variation of 0.2 cc. shows these effects.

The rate of the penetration of tissues by the fixative is increased by the alcohol, glacial acetic acid, and urea. Picric acid and formalin decrease the rate of the penetration; the small amount of mercuric chloride has no appreciable effect. The rapidity of the penetration of the tissues, and the solubility of the picric acid in the alcohol and the acetone fixatives decrease in the following order: acetone, methyl alcohol, ethyl alcohol, and isopropyl alcohol fixatives. The degree of lysing of hemoglobin by the solvents in the fixatives decreases in the order named: acetone, isopropyl alcohol, ethyl alcohol and methyl alcohol fixatives.

The penetration of the fixative is rapid during the first 4 hours but gradually decreases thereafter. In 4 hours the fixative penetrates between 1 mm. and 2 mm. In 16 hours there is complete penetration of blocks 5 mm. thick. The optimum time for adequate and uniform fixation is between 16 hours and 48 hours. Blocks 2 mm. thick are penetrated completely in 4 hours but fixation is inadequate in less than 8 hours. Cystic structures, such as eyes, become much distorted during fixation owing to the rapid removal of the water; this is obviated if a small incision is made in the wall of the cystic structure. This fixative is inadequate for the fixation of thick blocks of tissue in a manner in which Kaiserling's solutions and formalin are used. Large blocks of tissue are adequately fixed, however, if the thickness does not exceed 5 mm. The fixative may be used several times if 20 cc. of the fixative are used for 1 cc. of tissue.

Dehydration, Clearing and Paraffin Impregnation: The water content of the fixative is 10 per cent. Removal of water from the tissues and the fixative during dehydration presents little difficulty. An important action of the dehydrating agents is, however, the removal of the fixative from the tissues because an excess of retained fixative repels paraffin during impregnation of the tissues. Acetone is the dehydrating agent of choice in the rapid paraffin techniques owing to the rapid removal of the water and the fixative from the tissues. Ethyl

alcohol, absolute, is the dehydrating agent of choice in the extended paraffin techniques owing to the better maintenance of the fixation. Isopropyl alcohol 99 per cent is a satisfactory substitute for absolute ethyl alcohol. Its action is slower and there is less hardening of tissues. Tissues may be left in isopropyl alcohol (99 per cent) for longer periods without overhardening which occurs after 3 hours in acetone and 8 hours in absolute ethyl alcohol. Butyl alcohol and dioxan dehydrate more slowly than the ethyl and isopropyl alcohols. They are not recommended owing to the poor maintenance of the fixation. For an unknown reason, isolated areas in fat occasionally resist complete dehydration.

The most satisfactory clearing agent is aniline. Methyl benzoate, cedar oil and methyl salicylate are useful.

Paraffin impregnation may directly follow acetone dehydration, whereas an intermediate step is necessary or advisable following the other dehydrating agents. The intermediate agents replace the dehydrating agents in the tissues and, by virtue of their solvent effect on the paraffin, prepare the tissues for paraffin impregnation. The most useful intermediate agents are benzol and chloroform. The addition of paraffin to the solvents and their use at room temperature counteract their tendency to overharden tissue. Given identical conditions, the rate of paraffin impregnation of blocks of tissue depends on the degree of solubility of paraffin and the boiling point of the medium preceding immersion of blocks in paraffin. The solubility of paraffin decreases in the order named: xylol, chloroform, benzol, dioxan and acetone. The boiling points are: acetone 56°C., chloroform 62°C., benzol 80°C., dioxan 102°C., and xylol 140°C. Ideal combinations of a high degree of paraffin solubility and a low boiling point are possessed by chloroform and benzol. Paraffin is very slightly soluble in acetone, but acetone approaches the ideal owing to its boiling off or rapidly vaporizing at the temperature of the melted paraffin. An increase in the number of changes of paraffin is necessary following xylol and dioxan.

Difficulty in cutting adequately fixed tissues is usually the result of inadequate dehydration and paraffin impregnation of the tissues. The cut surface of the imbedded tissues is white, opaque and friable in areas of inadequate dehydration, whereas the uncovered cut surface becomes

concave within 24 hours in areas of inadequate paraffin impregnation. The quality of paraffin imbedded tissues is not impaired within 2½ years of storage.

Shrinkage and Distortion: Blocks of tissues show practically no shrinkage in volume during fixation. There is about 5 per cent shrinkage during dehydration and about 10 to 15 per cent shrinkage during paraffin impregnation. The total shrinkage in volume of tissue blocks is usually less than 20 per cent. Slow procedures incur less shrinkage than rapid procedures; the isopropyl-alcohol-benzol paraffin combination gives least shrinkage. The solvents, especially chloroform, produce shrinkage by removal of substance from tissue; the addition of paraffin tends to decrease this effect. Prolonged impregnation in paraffin incurs no additional shrinkage if the paraffin is not overheated. Similarly treated well preserved and autolyzed tissues shrink equally.

Shrinkage in volume associated with adequate paraffin impregnation, as described above, incurs minimal distortion of the tissue structures. On the other hand, inadequate paraffin infiltration is the greatest source of structural distortion if the fixation is adequate. Overhardening and insufficient dehydration of the tissues are the usual causes of inadequate paraffin impregnation. Excessive dehydration, fixation and drying of blocks cause excessive hardening. The impact of the microtome knife further distorts inadequately impregnated tissues by compressing the sections in the diameter perpendicular to the edge of the knife.

Staining Affinities: The ingredients of the fixative exert individual effects on the staining affinity of structures of tissues in regard to staining with acid and basic aniline dyes and with natural dyes, such as hematoxylin. Picric acid and mercuric chloride exert a general affinity for dyes whereas the remaining ingredients impart an affinity for acid aniline dyes on the one hand and basic aniline dyes and the natural dyes on the other hand. For instance, picric acid and mercuric chloride impart an affinity to the nucleus for basic fuchsin and hematoxylin and an affinity to the cytoplasm and the connective tissues for acid fuchsin and eosin. Formalin, glacial acetic acid and alcohol impart an affinity for basic aniline dyes and natural dyes and therefore accentuate staining of the nucleus. Urea imparts an affinity for acid

aniline dyes and therefore accentuates staining of cytoplasm and connective tissues. Furthermore, the ingredients exert individual staining affinities for different dyes in the same category. For instance, the Masson (10) trichrome stain uses three acid dyes: Ponceau dexylidine, acid fuchsin and Light Green or aniline blue. Picric acid, urea and mercuric chloride impart an affinity for the Ponceau dexylidine-acid fuchsin stain whereas formalin, acetic acid and alcohol impart an affinity for Light Green or aniline blue. Isopropyl alcohol imparts affinities for basic aniline dyes and natural dyes more strongly than ethyl alcohol. Tannic acid exerts a strong affinity for basic aniline dyes but not for hematoxylin, which stains poorly. Chromic acid exerts a strong affinity for acid dyes and also causes poor hematoxylin staining. Benzamide imparts an affinity for basic aniline dyes and natural dyes. The affinity of structures for dyes is accentuated by such factors as mordants, bleaches, buffers and the dye solvent but the fundamental affinities imparted by the fixative remain dominant.

The affinity of bacteria for dyes is selectively imparted by the ingredients of the fixative. In the Gram stain the alcohol imparts a strong affinity for the crystal violet and basic fuchsin whereas the other ingredients accentuate the affinity for one of the dyes. Formalin and mercuric chloride accentuate staining by crystal violet. Picric acid, glacial acetic acid and urea accentuate staining by basic fuchsin.

Refixing formalin and Kaiserling fixed tissues imparts affinities to structures similar to, but less pronounced than affinities imparted by primary fixation with the dehydrating fixative.

Prolonged fixation considerably decreases the general affinities of dyes for tissue structures. For instance, acid-fast bacilli are unstainable by basic fuchsin after fixation for 10 days and the bacilli are stained less easily after 3 days of fixation. Satisfactory hematoxylin and eosin stains are obtainable, however, after a fixation of 30 days.

Dehydrating and clearing agents, paraffin solvents and the degree of paraffin impregnation affect materially the brilliance and clarity of staining. Methyl alcohol, ethyl alcohol, acetone, aniline, methyl benzoate, benzol and xylol maintain or enhance the affinity for acid dyes without noticeably altering the affinity for basic aniline dyes and natural dyes. Isopropyl alcohol, butyl alcohol, dioxan, methyl salicylate

and chloroform decrease the staining affinity for acid aniline dyes but exert no noticeable effect on affinity for basic aniline dyes and natural dyes. In regard to the affinity for eosin, dioxan exerts the most harmful effect. Inadequate paraffin infiltration is the greatest factor in exerting a deleterious effect on the general staining affinities of adequately fixed tissues.

Isopropyl alcohol, butyl alcohol and dioxan are unsatisfactory substitutes for ethyl and methyl alcohols and acetone in the differentiation of stains owing to a deleterious effect on stains by acid dyes.

Glacial acetic acid inhibits slightly the staining of granules of eosinophile leucocytes. Picric acid has a similar effect on granules of basophile cells of the hypophysis. The staining affinity of certain bacteria, for example, B. tularensis and B. melitensis, is apparently lost in the tissues in which they lie, for the bacteria are easily stained in a suspension in 1 per cent agar, which is hardened, fixed and sectioned. Cultured spirochetes are similarly demonstrated. Although hemoglobin is lysed, red blood cell membranes are readily stained and morphological changes are demonstrable. Granules of eosinophile leucocytes and basophile cells of the hypophysis and red blood cells stain normally in refixed formalin and Kaiserling fixed tissues.

SUMMARY

A dehydrating fixative for general use is described. It is especially useful in the rapid preparation of sections for microscopic study. Tissue structures and bacteria are well preserved.

A wide range of routine and special stains is applicable. New stains and techniques for use with the fixative are described and discussed.

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U. S. ARMY GENERAL HOSPITALS 18 AND 118 ORGANIZED AT THE JOHNS HOPKINS HOSPITAL

In response to a request from the Surgeon General of the Army that The Johns Hopkins Hospital organize General Hospital No. 18 as a successor to Base Hospital No. 18, which served in the last World War, the hospital authorities agreed and undertook the organization of the General Hospital No. 18, with a capacity of 1,000 beds. Dr. George G. Finney, son of the late General John M. T. Finney was chosen as Chief of the Surgical service and Acting Director of this affiliated unit, and Dr. James Bordley, III was chosen as Chief of the Medical service. Dr. Finney and Dr. Bordley proceeded with the organization and the unit was completed, when about the first of April it was learned that the Surgeon General of the Army felt that smaller units would be much more desirable. Accordingly, The Johns Hopkins Hospital offered to organize two General Hospitals of 500 beds each instead of the one of 1,000 beds if that would better meet the current demands of the Army. A similar offer was made by the University of Maryland. The offer was accepted and the result was the completed organization of General Hospital 18 and General Hospital 118.

About the middle of April both units were called into service. A few days before their departure the Trustees and Officers of The Johns Hopkins Hospital held a reception in honor of the officers and nurses composing the two units. At the reception brief exercises were held at which Dr. Winford H. Smith, the Director of The Johns Hopkins Hospital, presided, and gave a brief history of the organization of Base Hospital 18 and its service in the last war, and reviewed the steps which led up to the creation of the two general hospitals for service in the present war. Dr. William A. Fisher, Jr., who was a member of the staff of Base 18, and for a time, after General Finney was detached to become Chief Surgical Consultant for the A.E.F., served as Chief Surgeon of Base 18, presented to Colonel George Finney and Colonel James Bordley a United States flag and a Johns Hopkins flag for each of the two new units. He also presented two atlases to each unit, one for the medical officers and one for the nurses, in order that the members of the unit might know exactly where they were geographically

and be able to follow events accurately on the map. Dr. Fisher reviewed briefly the record of Base 18 in World War No. 1 and expressed the belief that the new General Hospitals 18 and 118 would be worthy successors and live up to the splendid traditions of The Johns Hopkins Hospital.

Mr. John Sears Gibbs, Jr., President of the Board of Trustees, then presented to Colonels Finney and Bordley a sum of money which had been contributed by friends of the Hospital and of the doctors and nurses connected with the hospital for their use anywhere, any time, for their greater comfort, pleasure or efficiency. Mr. Gibbs stated that a single letter had been sent out to a limited number of individuals, including the Board of Trustees, the Women's Auxiliary Board, members of the staff not in the Unit and a very few friends outside of the officers' families and the response had been prompt and most generous. He turned over to each unit a sum of $5,000. After these brief exercises the reception continued. There were about eight hundred people present.

Both units left Baltimore for camps in this country the same night, one early in the evening, and one two or three hours later. The scene at the station when they departed was impressive. Probably two hundred and fifty or three hundred friends of the medical officers and nurses were present. A colored instrumental quartet played popular music and popular songs were sung and the departure of these two units was made a pleasant although serious occasion.

Both of these units are now on foreign soil and it is the confident expectation of the trustees and officers of the Johns Hopkins Hospital that they will render a good account of themselves no matter what may develop.

The personnel of the two units is as follows:

GENERAL HOSPITAL NO. 18

Lt. Colonel Amos Koontz, Commanding
Lt. Colonel Benjamin M. Baker
Lt. Colonel George G. Finney

Major Richard C. Tilghman
Major Alexander J. Schaffer
Major Ralph G. Hills

Major Richard T. Shackelford
Major H. Alvin Jones
Major Dudley C. Babb
Major Adam C. Bock
Major Webster H. Brown

Captain Theodore Lidz
Captain Walter B. Buck
Captain Edward Nichols
Captain M. Richard Whitehill
Captain M. Louis Singewald
Captain Edward S. Stafford
Captain C. B. Troland
Captain William S. Watson
Captain Douglas H. Stone
Captain Edmund B. Kelly
Captain Hayward B. Streett
Captain Mitchell H. Miller
Captain C. Bernard Brack

Lieutenant Max Michael
Lieutenant Phillip A. Tumulty
Lieutenant William C. Quinby, Jr.
Lieutenant Paul Higgins
Lieutenant R. L. Payne, Jr.
Lieutenant James E. T. Hopkins
Lieutenant Roger A. Lewis
Lieutenant Gustav Kruger

Captain George Blakiston
Lieutenant Carroll D. Hill
Lieutenant John A. Beckett
Lieutenant Herbert M. Carnahan
} Administrative Assistants

NURSING PERSONNEL—GENERAL HOSPITAL NO. 18

Amelia Jane Pierson, Chief Nurse

Marie M. Ames
Clara W. Bauer
Alice L. Black
Virginia L. Bonney
Eleanor P. Cassidy
Susanna L. Chase
Frances E. Childress
Rachel H. Cook
Frances H. Daws
Vivian V. Demarest
Isabelle C. Diehl
Daphine Doster
Frances Faith
Charlotte M. Fischer

Mary Elizabeth Geddes
Pearl D. Graham
Evelyn Hamilton
Georgia Hardesty
R. Jean Hays
Lillian M. Helbig
Anna E. Holmes
Margaret Huger
Evelyn Hulse
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Mattie L. Kilby
Virginia Brown Lester
Marion L. Lowe
Louise Ludwig
Lilyan W. Marshall
Josephine Mersonake
M. Elizabeth Miller
Josephine Mills
Sara E. McAllister
Martyne McComb
Elizabeth K. McLaughlin
Elizabeth McNamara
Ethel Niemi
Mary A. Patterson
Ruth H. Reynolds
Marjorie M. Riblet
Dorothy H. Rook
Florence E. Rothe
Barbara Siegel
Helen McBride Smith
Elinor Stearns
Pauline Stecker
Grace Sterns
Jean Vincent
Marianne R. Welker
Elizabeth Whitaker
Mary Wochley
A. Elizabeth Wright
Alice E. Adcock
Annie Mary Donnell
Nancy B. Kinsey
Mary McClain
Agnes B. McGann
Anne V. Papay
Ruth Podboy

GENERAL HOSPITAL NO. 118

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Lt. Colonel James Bordley, III
Lt. Colonel I. Ridgeway Trimble

Major Walter L. Winkenwerder
Major Thomas McP. Brown
Major Paul A. Kunkel
Major George O. Eaton
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Major J. Herman Long
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Captain Henry M. Fox
Captain F. Tremaine Billings
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Captain Edward T. Newell, Jr.
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Lieutenant John S. Hunt
Lieutenant John B. Tredway
Lieutenant Allan B. Warren
Lieutenant William N. Jones
Lieutenant William E. Grose
Lieutenant Eugene D. Lyon
Lieutenant Wilbur Burton
Lieutenant William R. Bethea, Jr.
Captain Edwin H. Webster, II
Captain Robert J. Moore
Captain George McLean Shriver, Jr.
Captain John Whitridge
} Administrative Assistants

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Marguerite Sourneuf
Vesta Boyd
Harriett A. Brown
Mary A. Brun
Alta M. Bumpus
Hazel Burke
Florence Butler
Emma H. Chapman
Irene V. Chitsey
Ruth A. Dale
Helen M. Dolan
Verna Doring
Marguerite L. Evans
Mary E. Farr
Anna L. Forrest
Marian F. Fox
Mary Agnes Gautier
Florence M. Hack
Candace L. Heinley
Edith Hoffman
Louise Hohn
V. Marie Hontz
M. Isabel Harris
Katherine Irby
Esther Jacoby
Bernice Janov
Eva L. Kelly
Cecille M. King
Florence King
Ruth Klingelhofer
Eleanor Lanham
Elizabeth Mackevrican
Dora Mansfield
Cecilia A. McMahon
Nancy L. Muhleman

Martina Nelson
Mary M. Newcomer
Eleanor S. Niernsee
Edith A. Nunn
Mary E. O'Connor
Bertha K. Philips
Virginia D. Roach
Anne H. Seney
Florence E. Smith
Thelma Shawley
Berla M. Smythe
Doris L. Snyder
Virginia J. Thompson
Virginia Varney
Helen J. Weber
Anna E. West
Margaret E. Wheeler
Dorothy Best
Muriel Cuggolz
E. Elaine Hess
Dorothy E. Darling
Bertha Mellon
Catherine Mellon
Marian E. Nichols

BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal.)

The Doctors Mayo. By HELEN CLAPESATTLE. 822 pp. Illus. $3.75. *University of Minnesota Press, Minneapolis, 1941.*

> And he who in our daily sight
> Seems but a figure mean and small,
> Outlined in Fame's illusive light,
> May stalk, a silhouette sublime,
> Across the canvas of his time.

This is one of the great biographies. Written by one who has been so closely attached to the clinic it is surprisingly modest and objective. That the two Mayo brothers starting with their father, a very interesting and virile personality, as ordinary general practitioners, should have built in a small country town in the middle west one of the greatest medical institutions in the world, and in such a brief period, is perfectly amazing. It is one of the American epics. Founded upon the simple principle (from which they never wavered) that the patient's interests were always paramount and that they should be provided with the best that surgery and medicine could offer, the response quickly magnified the simple institution into an enormous clinic. Lesions operated upon by others in small numbers, were soon reported by the Mayos in hundreds and even thousands of cases, and always with superior results. A constant trek of the world's foremost surgeons to this Mecca in the wilderness was perhaps the finest silent tribute to the quality of their work.

Their success was in large part due to their constant search for improvements in diagnosis, operative procedures and technique. While one of the Mayos was working at home the other was investigating the newer developments in surgery elsewhere. And as the clinic grew larger traveling to other centers was equally expected of the younger men. Until their deaths the Mayo clinic continued in this progressive stride.

Although Dr. Will Mayo had the more conspicuous part in the organization and development of the clinic, Dr. Charlie contributed his share and was an ideal complement to his brother. Neither could have created it alone. Although Dr. Will always insisted that the period in which they lived was the most important item in their success (and it certainly was important), nevertheless the same opportunity was open to all and even more to the large medical schools and hospitals, but it was the Mayo brothers who seized the opportunity and created a masterpiece. No effort is made to place the Mayos among the great contributors to science by original investigations, but they certainly contributed much to the care of patients and to the development of the high specialization that has now

become outstanding in modern medicine and surgery. Moreover, they filled the country with well trained surgeons, many of whom started miniature "Mayo clinics".

The efforts of the Mayos to obtain affiliation with the University of Minnesota and, therefore, afford University recognition for the clinic is an interesting part of the biography. Although their offer of $1,500,000.00 (subsequently increased) had almost no strings attached and gave the University far more than could be expected in return, the gift was bitterly fought by the legislature and even by some of the University trustees, prompted by petty jealousies. Finally wiser counsel prevailed, with marked benefit to both.

There are few biographies that the reviewer has read that have been so entertaining and at the same time so revealing of the human qualities that produce success in life. To ambitious medical students and young graduates it is especially commended.

W. E. D.

Surgery of Head and Neck. By ARTHUR S. MCQUILLAN. 138 pp. $2.00. *Oxford University Press, London, New York, Toronto, 1941.* (Oxford Medical Outline Series.)

This is an excellent little textbook of lesions of the head and neck, in outline form. Lesions of the brain are not included. Etiology, signs, symptoms, diagnosis, differential diagnosis and treatment are tabulated in one, two, three order and with surprising accuracy and unusual thoroughness. This condensed form is particularly valuable for the medical student to whom an orderly and brief enumeration of the important desiderata in diagnosis and treatment is most helpful. The chapter on fractures of the skull gives the student a much saner view of this subject than can be obtained in the volumes of misleading writings that are now in print.

The bibliographic documentation is not too well selected, and for a text of this character would perhaps better have been omitted.

W. E. D.

Treatment of Burns. By HENRY N. HARKINS. 457 pp. Illus. $6.50. *Charles C. Thomas, Springfield, Illinois, 1942.*

An excellent monograph on burns has been published at a most opportune time. The book is not confined to the treatment of burns as the title might imply, for many interesting historical aspects are discussed, there are sections dealing with the chemistry and pathology of burns and of shock, and the rôle of the adrenals, the complications of burns, and also plastic reconstructive surgical procedures are considered.

The various types of burns are discussed, with special sections on war burns, industrial burns, and those due to radiation, chemicals and freezing. The work is carefully documented and contains about 1300 references. It is at the same

time eminently practical, for Dr. Harkins has successfully correlated experimental studies with extensive clinical practice. The various methods of treatment are carefully evaluated with a realization that the final conclusions and the ideal therapeutic agent for the treatment of burns has not yet been found.

This book can be highly recommended and is of especial importance at the present time.

K. L. P.

Clinical Immunology, Biotherapy, and Chemotherapy in the Diagnosis, Prevention and Treatment of Disease. By JOHN A. KOLMER and LOUIS TUFT. 941 pp. Illus. $10.00. *W. B. Saunders Company, Philadelphia and London, 1941.*

As the title implies this book is a comprehensive monograph on infectious disease. The authors discuss the principles of bacteriology and immunology and their application not only to bacterial disease but to virus diseases and parasitic infections as well. The reviewer's principle criticism of this volume is that the attempt is made to cover so broad a field that the consideration of many of the more common infections encountered in medical and surgical practice is superficial. In spite of this weakness, the authors have presented excellent discussions of certain phases of infectious disease. The treatment of the allergies, for example, is clear and concise. In the discussion of specific diseases, the authors have failed in certain instances to emphasize the pathogenesis of the infection and, thereby, have failed to clarify the rationale of therapy. Too often they have been content to list the results of therapy without attempting to explain failures or to suggest possible improvements in therapeutic methods. Finally they have made rather startling statements in the text without reference to the original source. For example, they state that "the results of experiments indicate that the histiocytes can produce antibodies in the fully formed state." Not only would many immunologists challenge such a statement, but they would certainly desire a reference to the original experiments supporting this conclusion.

In spite of the numerous criticisms which may be made of the monograph, it serves a valuable purpose in bringing to the attention of students and practitioners the more modern methods of treating infectious disease. Its publication is timely because of the importance of the subject to military medicine.

W. B. W., JR.

Effective Living. By C. E. TURNER and ELIZABETH MCHOSE. 432 pp. Illus. $1.90. C. V. Mosby Company, St. Louis, 1941.

This book covers a wide range of hygiene. The first part deals with effective living for the individual, the second with effective living in the family, and the third with effective living in the community. The book is written as a text-book for use at the high-school level, and should serve its purpose very well. Much information is given by the well selected and numerous illustrations.

The book is not, however, of particular interest to medically trained readers,

although it should be useful to those who wish to acquire accurate knowledge in a simple form about the human body, how it functions, and how to keep it in a healthy, effective state.

G. C. R.

Lachish II. The Fosse Temple. By The Wellcome-Marston Archaeological Research Expedition to the Near East. 104 pp. 73 pl. $8.50. Oxford University Press, New York [etc.], 1940.

In 1932, on the suggestion of the British archeologist, J. L. Starkey (d. 1938), excavations were begun at Tell ed Duweir, probably the site of the old Amorite town of Lachish. The excavations led to the gradual discovery of an ancient temple of three superimposed structures. This temple was erected in a "fosse" outside the original city walls. Its construction was probably begun in the early 15th century B.C. and the building was destroyed approximately 300 years later. A well illustrated description and analysis of this building, as well as of objects found in and around it, form the main part of the book.

Quite a few animal bones were discovered, probably the remainders of offerings similar to those described in Leviticus III, 1–17 and VII, 15–18, 29–34. A preliminary and tentative analysis by Professor Watson of University College, London, had the following results (p. 93): "Four types of animals are represented, sheep (or goat), ox and two wild beasts such as gazelle or ibex. There are also bird and fish bones, including teeth of the eagle-ray. Two remarkable features are observed, that the animals are all very young, and practically all the identifiable bones are metacarpals of the right foreleg. This applies to the bones from all these structures." The great number of "knucklebones" near an altar suggests to the authors the possibility of their use for divination.

O. T.

BOOKS RECEIVED FOR REVIEW

Clinical Hematology. By MAXWELL M. WINTROBE. 792 pp. Illus. $10.00. *Lea & Febiger, Philadelphia, 1942.*

Four Treatises of Theophrastus von Hohenheim, called Paracelsus. Translated from the original German, with Introductory Essays by C. LILIAN TEMKIN, GEORGE ROSEN, GREGORY ZILBOORG, HENRY E. SIGERIST. Edited, with a Preface by HENRY E. SIGERIST. 256 pp. $3.00. *The Johns Hopkins Press, Baltimore, 1941.*

Anoxia: Its Effect on the Body. By EDWARD J. VAN LIERE. 269 pp. Diagrams. $3.00. *University of Chicago Press, Chicago, 1942.*

Manual of Pharmacology. By TORALD SOLLMANN. 6th ed. 1298 pp. $8.75. *W. B. Saunders Company, Philadelphia and London, 1942.*

Neural Mechanisms in Poliomyelitis. By HOWARD A. HOWE and DAVID BODIAN. 234 pp. Illus. $3.50. *The Commonwealth Fund, New York, 1942.*

Diseases of Metabolism. Edited by GARFIELD G. DUNCAN. 985 pp. Illus. $12.00. *W. B. Saunders Company, Philadelphia and London, 1942.*

The Conquest of Bacteria from Salvarsan to Sulphapyridine. By F. SHERWOOD TAYLOR. 178 pp. Diagrams. $2.00. *Philosophical Library and Alliance Book Corporation, New York, 1942.*

Communicable Disease Nursing. By THERESA I. LYNCH. 678 pp. Illus. $3.75. *The C. V. Mosby Company, St. Louis, 1942.*

Rabies. By LESLIE T. WEBSTER. 168 pp. Illus. $1.75. *The Macmillan Company, New York, 1942.*

Surgical Physiology. By JOSEPH NASH. 496 pp. Illus. $6.00. *Charles C. Thomas, Springfield, Illinois, 1942.*

Home Guide to Modern Nutrition. By N. D. PHILLIPS. 96 pp. 50 cents. *Longmans, Green and Co., New York & Toronto, 1942.*

Ornithologists of the United States Army Medical Corps. By EDGAR ERSKINE HUME. 583 pp. Illus. $5.00. *The Johns Hopkins Press, Baltimore, 1942.*

Textbook of Clinical Parasitology. By DAVID L. BELDING. 888 pp. Illus. $8.50. *D. Appleton-Century Company, New York, 1942.*

THE ROLE OF HYPERSENSITIVITY IN PERIARTERITIS NODOSA

As Indicated by Seven Cases Developing During Serum Sickness and Sulfonamide Therapy

ARNOLD RICE RICH

From the Department of Pathology and Bacteriology, The Johns Hopkins Medical School, Baltimore, Md.

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Before the introduction of sulfonamide therapy it was a great rarity for a case of serum sickness to come to autopsy in this hospital. Antiserum was administered on the wards mainly to patients with pneumonia or meningitis and, with rare exceptions, the patients either failed to develop serum sickness, or they died before a lapse of time sufficient to permit serum sickness to develop, or else the serum was effective in overcoming the infection and if serum sickness occurred the patients did not die. Death from serum sickness, itself, is exceedingly rare. Longcope and Winkenwerder (1), in their review written in 1941, stated that no fatality had been reported up to that time.

Since sulfonamide therapy has been in use, autopsies on patients with serum sickness, or who had had serum sickness shortly before death, have been more frequent, for the reason that even in ultimately fatal cases of infection the sulfonamides, used in conjunction with antiserum, may prolong life sufficiently to permit serum sickness to occur before the patient dies from the infection. Under these conditions there have come to autopsy in this department within a period of 9 months, 4 cases in which manifestations of serum sickness developed during the terminal illness.

Of course, in patients who are desperately ill from the infection for which the antiserum is administered, as was the case in the present instances, the entire clinical picture of serum sickness cannot be as clear-cut as in cases in which the condition appears during convalescence. Fever is present from the infection itself, and nausea and albuminuria may be referable to the same cause; the leucocytosis of infection can mask the tendency of serum sickness to cause leucopenia;

and desperately ill patients may not complain of joint pains. In the present cases, there was a skin rash which was described as urticarial in three, and as "erythematous, almost papular" in the fourth. In two cases marked general glandular enlargement appeared; in the other two there was no mention of the condition of the lymph nodes. In two cases (Autopsies 17250 and 17291), the urticaria and general glandular enlargement appearing on the seventh and eighth day following the first administration of serum were diagnosed clinically as manifestations of serum sickness. In a third case (Autopsy 17257) the patient had repeated mild reactions to the serum injections on the first day (dyspnoea, cramps), and developed urticaria on the second day during further treatment; in the fourth case (Autopsy 17628) the "erythematous, almost papular," rash that appeared on the eighth day following the injection of serum and sulfadiazine was regarded by the observer as probably having been due to the drug. The rash of serum sickness may be indistinguishable from that caused by the sulfonamides. Both may be urticarial, erythematous, or papular. Evidence that the sulfonamide eruptions are manifestations of hypersensitivity will be mentioned below.

Because of the infrequency of autopsies on patients with serum sickness, the first of the present cases was examined with particular interest, and it was with surprise that fresh lesions of periarteritis nodosa were encountered. This was regarded as being probably only a coincidence until, shortly afterwards, the second case that had had an urticarial serum reaction came to autopsy, and again marked lesions of periarteritis nodosa were present. These cases were presented at a clinical-pathological conference in March 1941, with the suggestion that, since periarteritis nodosa is no common disease, the combination of serum sickness with the arterial lesions in patients who had been well before the fatal lobar pneumonia for which they were treated might be more than a coincidence. Shortly afterwards, the third case diagnosed clinically as having had serum sickness during the terminal illness came to autopsy and, again, widespread, fresh lesions of periarteritis nodosa were found; and this was followed by a fourth case in which the same type of vascular lesion was found at autopsy. Clinical interest was aroused in the matter by the presentation of these various cases at clinical-pathological conferences as they appeared,

with the result that when a fifth patient (S.P. 81125, Table I), who had received antiserum for pneumonia, developed serum sickness (skin rash and enlargement of epitrochlear lymph nodes) followed by symptoms suggestive of periarteritis nodosa (pains in muscles, haematuria, impairment of renal function, development of hypertension) a biopsy was performed, and in the small piece of biceps muscle tissue obtained there was found a marked periarterial inflammatory infiltration of polymorphonuclear and mononuclear cells (Fig. 7). This patient was discharged, three months after admission, with hypertension and with a persisting slight diminution in renal function.

All five of the above-mentioned patients had been treated not only with foreign serum but also with sulfonamides, and the question therefore arose as to the possible rôle of the drugs in causing the vascular lesions. One additional case indicates that sulfathiazole may, under certain conditions, produce lesions of this type. This patient (Autopsy 17529, Table I) was a negro of 73 years, who had carcinoma of the pancreas. He vomited continually while under anesthetic in preparation for an exploratory laparotomy. The operation was not performed, and the patient was returned to the ward and was given repeated doses of sulfathiazole orally and intravenously as a prophylactic measure against aspiration pneumonia. Death occurred a week later, and at autopsy there were arteries in the sections of the kidneys and of a cervical lymph node which showed the hyaline necrosis and perivascular infiltration with mononuclear and polymorphonuclear cells characteristic of periarteritis nodosa (Figs. 11, 12, 13). In addition, there were widespread focal parenchymal lesions in the viscera (necrosis, infiltration of inflammatory cells) precisely like those recently described by Lederer and Rosenblatt (2) in four patients who died during sulfathiazole medication. The latter authors made no mention of vascular lesions. No bacteria could be stained in these inflammatory-necrotic foci, and smears and cultures of the heart's blood and lungs were negative.

Only one of the above five patients treated with serum had received sulfathiazole before the rash appeared. Sulfadiazine was the only sulfonamide which had been given to the other four patients before the rash appeared, with the exception of one who received 2 grams of sulfapyridine on the first day and sulfadiazine thereafter. That serum

TABLE I

Summary of Clinical Data and Pathological Findings

AGE, SEX, RACE, AUTOPSY NO.	INFECTION FOR WHICH SERUM WAS GIVEN	AMOUNT AND CHARACTER OF SERUM*	AMOUNT AND TYPE OF SULFONAMIDE	TIME OF APPEARANCE OF HYPERSENSITIVE REACTION	HYPERSENSITIVE REACTION	DAYS LIVED AFTER ONSET OF HYPERSENSITIVE REACTION	PAST HISTORY†	PATHOLOGICAL FINDINGS	
								Sites of periarteritis nodosa	Other lesions
37, M., C. 17250	Lobar pneumonia (Type VIII)	900,000 units rabbit serum on 2nd and 3rd days in hospital. 880,000 units horse serum on 7th and 8th days.	4 gms. sulfadiazine i.v. 1st day in hospital. 4-6 gms. per day orally next 7 days	8 days after first serum; 9 days after first sulfadiazine.	Urticaria; marked general glandular enlargement.	5 days	P.N. neg. A. neg.	Kidney, renal pelvis, testis, lung, peripancreatic tissue.	*Necroses in lymph nodes; Myocarditis (polys, mones, eos.);* organizing pneumonia; nodular cirrhosis; syphilitic aortitis; thrombus in aorta; renal infarcts; diphtheritic colitis.
23, F., W. 17257	Lobar pneumonia (Type I)	1,800,000 units (700 cc.) horse serum on 2nd–3rd day in hospital. 1,000,000 units (235 cc.) rabbit serum on 3rd–4th day.	5–6 gms. sulfadiazine 1st–23rd day in hospital.	Frequent mild reactions (dyspnoea, cramps) during the injections of horse serum. Urticaria on next day.	Marked urticaria. Lymph nodes not mentioned.	20 days	P.N. neg. A. no mention.	Heart, liver, renal pelvis.	*Myocarditis (polys, mones, eos.);* pulmonary abscesses; organizing pneumonia; broncho-pleural fistula; empyema.
21 mos., F., W. 17291	Meningitis (H. influenzae)	120 cc. horse serum i.v. on 12th–17th day in hosp. 135 cc. intrathecally on 12th–25th day.	2 gms. sulfapyridine 1st day in hosp. 3–7.5 gms. sulfadiazine on 2nd–14th day.	On 12th day of sulfadiazine a maculopapular rash. Drug discontinued, rash faded. Urticaria on 7th day after first serum.	Urticaria and marked general glandular enlargement following serum. Maculopapular rash following sulfadiazine.	11 days after onset of serum sickness. 15 days after sulfadiazine rash.	P.N. neg. A. no mention.	Ovary, uterus, renal pelvis, lung, bladder, meninges, hypophysis.	*Necroses in lymph nodes; myocarditis (polys, mones. eos.);* organizing meningitis; cavernous sinus thrombosis; pneumonia; ascaris infestation.
49, M., W. 17628	Lobar pneumonia (Type IV)	960,000 units (150 cc.) rabbit serum on 1st–2nd day in hospital.	5 gm. sulfadiazine i.v. on 1st–2nd day in hosp. 3–12 gms. per day orally on 3d–9th day. 2–4 gms. sulfathiazole on 9th–10th day. 5–6 gms. sulfapyridine on 11th–13th day.	8 days after first serum; 8 days after first sulfadiazine; some hours before sulfathiazole; 2 days before sulfapyridine.	"Erythematous, almost papular eruption". Lymph nodes not mentioned.	9 days	P.N. neg. A. no mention.	Testis, liver, pancreatic duct.	Pulmonary abscesses; organizing pneumonia; empyema; ac. prostatitis; chr. cystic pancreatitis with calculi.

37, M., W. Biopsy, S.P. 81125	Lobar pneumonia (Type I)	600,000 units rabbit serum on 2nd day in hospital. 1,000,000 units on 3d day. 100,000 units on 4th day.	6–9 gms. sulfadiazine per day on 1st–9th day in hospital. 2–6 gms. sulfathiazole on 9th–14th day. 3–6 gms. sulfadiazine on 17th–27th day.	10 days after first serum; 3 days after first sulfathiazole; 12 days after first sulfadiazine.	Punctate, erythematous rash over chest, arms, abdomen and back. Epitrochlear nodes enlarged.	Alive. Biopsy taken 4 weeks after hypersensitive reaction.	P.N. neg. A. neg.	Muscle (biopsy).	
39, M., W. 14664	Meningitis (H. influenzae)	80 cc. horse serum i.v. and 30 cc. intrathecally on 1st and 2nd day in hosp. Total of 58 cc. intrathecally 7th day to death.	None	5 days after first serum.	Urticaria. Lymph nodes not mentioned.	9 days	P.N. neg. A. no mention.	Renal pelvis, meninges.	Meningitis; lobular pneumonia.
73, M., C. 17529	Sulfathiazole given prophylactically to forestall aspiration pneumonia following vomiting from anaesthesia	None	4–6 grams sulfathiazole orally for 4 days. 8–12 gms. i.v. per day for next 4 days.		None mentioned.		P.N. neg. A. no mention.	Kidney, lymph node.	*Inflammatory-necrotic foci in lungs, spleen, marrow, lymph nodes, kidneys, testis, retroperitoneal fat* (see text); cancer of pancreas; metastases in liver and lymph nodes; obstruction bile and pancreatic ducts; biliary cirrhosis; atrophy of pancreas.

* P.N. = symptoms suggestive of periarteritis nodosa before entering hospital.
A. = past history of asthma, hay fever or other allergic conditions.

sickness in the absence of sulfonamide therapy may be accompanied by the lesions of periarteritis nodosa is indicated by Autopsy 14664, Table I. This is the only autopsy on a patient who had had serum sickness shortly before death, in the period before the introduction of sulfonamide therapy, that we have been able to find either in the records of this department or in the files of the hospital History Room, which were kindly searched for this purpose by Miss Blanche F. Pooler, the Record Librarian. It was, therefore, of particular interest that a study of the sections of this case revealed vascular lesions like those of periarteritis nodosa. The lesions consisted of fresh fibrinoid and hyaline necrosis of segments of the walls of arteries of the renal pelvis and meninges, with a moderate infiltration of inflammatory cells.

The vascular lesions in all of the above-mentioned autopsies are those familiar in periarteritis nodosa, and need not be described in detail individually. Briefly, there is necrosis, fibrinoid alteration, and hyalinization of the media of the affected arteries, together with perivascular infiltration, or infiltration of the entire wall, with mononuclear and polymorphonuclear leucocytes. Eosinophils are present in large numbers in four cases (Autopsies 17250, 17257, 17291, 17628); they are less numerous in two cases (17529, 14664); and they are absent in the lesion found in the biopsy of the muscle taken 1 month after the onset of the serum sickness. There is hemorrhage about affected vessels in four cases (17250, 17291, 17628, 14664). The lesions are quite indistinguishable from those in the ordinary case of periarteritis nodosa of undetermined etiology. In some of the present cases the arterial lesions are widespread; in others, they are less numerous. Figures 1 to 13 illustrate representative lesions, and Table I lists their distribution and other pertinent clinical and pathological information relating to the individual cases.

DISCUSSION

In the consideration of the etiology of the vascular lesions in the present cases, the first question that arises is whether the presence of periarteritis nodosa in these patients was merely a coincidence, and unrelated to the terminal illness. While it cannot be proved not to have been a coincidence, the available evidence strongly indicates a relationship between the hypersensitive reactions and the vascular lesions.

In the first place, none of these seven patients had had symptoms suggestive of periarteritis nodosa before entering the hospital for the treatment of their terminal illness, which in all cases was an acute infection with abrupt onset, except in the case of the patient with carcinoma of the pancreas (Autopsy 17529).

In the second place, the vascular lesions in all except two of these cases were decidedly fresh,—a fact which indicates that they had developed shortly before death. In one case (Autopsy 17257) fibroblastic proliferation was more prominent in the inflammatory reaction about the vessels, and this particular patient had lived for three weeks after the appearance of the urticaria. In the other case in which fibroblasts were prominent (Biopsy 81125) the biopsy was obtained four weeks after the onset of the serum sickness.

Since the evidence indicates that the vascular lesions developed during the acute terminal infection for which the patients entered the hospital, it would seem quite improbable that six cases of periarteritis nodosa, of an etiology entirely unrelated to the terminal illness, developed successively on five different wards of this hospital during a period of 9 months, and that the only other prior case of serum sickness in our autopsy files likewise developed the very fresh lesions of periarteritis nodosa as a coincidence while in the hospital during his terminal acute illness several years ago.

If the vascular lesions in these cases were related to the terminal illness, as the evidence indicates, were they related to the infection for which the patients were treated, or to the serum therapy, or to the sulfonamide therapy?

It seems improbable that the lesions were the result of a direct action of the infecting bacteria or their products. The pneumococcus and the influenza bacillus were the only bacteria responsible for the infections for which the patients were treated, and certainly past experience in this and other departments of pathology has revealed no tendency for periarteritis nodosa to be associated with the large number of cases of these infections which come to autopsy. Clark and Kaplan (3) made an especial search for vascular lesions of this type in 52 cases of lobar pneumonia, and found none.

In regard to the possibility that the lesions can result from the hypersensitive reaction of serum sickness independently of sulfonamide therapy, the following facts are strongly persuasive:

First, the lesions were found in the viscera of all of the four cases of serum sickness that came to autopsy during the brief period of nine months, and, in addition, in the biopsy from the fifth patient who recovered from serum sickness during the same period.

In the second place, while these five patients had also received sulfonamides, the urticaria and general glandular enlargement in case 17291 seems rather clearly to have been due to the serum, for these manifestations of serum sickness appeared 7 days after the first injection of the serum and four days after the sulfonamide had been discontinued. Furthermore, a maculo-papular rash which had appeared on the twelfth day of sulfonamide therapy, and which was very probably a sulfonamide rash, faded after the discontinuance of the drug and before the urticaria and general glandular enlargement appeared. Case 17257, likewise, seems clearly to have been an instance of serum sickness rather than of drug hypersensitivity, for the patient had repeated mild hypersensitive reactions (attacks of dyspnoea and cramps) during the serum injections, and developed marked urticaria within 24 hours.

In the third place, the lesions were found in the single fatal case of serum sickness available in our files before the advent of sulfonamide therapy (Autopsy 14664).

Finally, it is of particular interest that an examination of the very meagre literature on the pathology of serum sickness revealed that Clark and Kaplan (3), in 1937, had noted arterial lesions of precisely the same character in two autopsies on patients who had had serum sickness shortly before death. In reply to an inquiry, these authors have informed me that neither of their patients had received sulfonamides in any form. It is noteworthy that in three of the present cases (Nos. 17250, 17257 and 17291) there was an infiltration of the myocardium by mononuclear and polymorphonuclear cells, including eosinophils, quite like that found by Clark and Kaplan (3) in their cases of serum sickness. The significance of this finding will be discussed below.

The occurrence of fresh lesions of the periarteritis nodosa type in these three cases of serum sickness (Autopsy 14664 and Clark and Kaplan's two cases), in patients who were well before the terminal acute infection for which serum was administered, and who had re-

ceived no sulfonamide therapy, is persuasive evidence that vascular lesions of this type can be a result of the anaphylactic type of hypersensitive reaction which is responsible for serum sickness.

There is, indeed, experimental evidence that vascular lesions of this general type can result from anaphylactic hypersensitivity. It is well known that vascular damage is a prominent feature of the local anaphylactic reaction of the Arthus type (4). Necrosis of the vessels, with adventitial inflammation, was described in Gerlach's study of the histology of the Arthus phenomenon twenty years ago (12). Pagel (13), likewise, has described necrosis and hyalinization of the smaller arteries at the site of the Arthus reaction, with adventitial infiltration by inflammatory cells. Several investigators have described the occurrence of arterial lesions in the viscera following the intravenous or subcutaneous injection of foreign protein into sensitized animals. Lesions showing a decided similarity to periarteritis nodosa in such experiments are described and illustrated by Masugi and Sato (16) and by Vaubel (5), who comment upon the close resemblance of the lesions to those in human periarteritis nodosa.

While the above evidence provides good reason to believe that periarteritis nodosa can be a manifestation of the anaphylactic type of hypersensitive reaction to foreign protein, it need not follow that the vascular lesions will occur in all cases of serum sickness. In the two cases of serum sickness of Clark and Kaplan (3) in which the lesions occurred, large amounts of serum had been given. The amounts given in the present series are shown in Table I. Kellett (6) and Fleischer and Jones (7) reported some years ago that a condition similar to human serum sickness can be produced in guinea pigs and rabbits by the injection of relatively large amounts of foreign serum, but no study of the tissues was mentioned. Experiments directed toward this end are in progress in our laboratory.

In regard to the possibility that lesions of the periarteritis nodosa type may be caused by the sulfonamides, Autopsy 17529 is strongly indicative that this may occur, for in this case these were typical fresh lesions in a patient from the surgical service who had received no serum, but who had been given repeated intravenous injections of sulfathiazole prophylactically to prevent aspiration pneumonia. There was no infection at autopsy. The patient was a negro, and no skin rash is

mentioned in the history. As was stated above there were present, in addition to the vascular lesions, widespread visceral focal, inflammatory-necrotic lesions of the character described by Lederer and Rosenblatt (2) in patients dying after sulfathiazole medication. These peculiar focal lesions seem rather clearly to be an effect of sulfathiazole. No bacteria were demonstrable in stains or by culture. Dr. Robert C. Crawford has shown the present writer two additional cases with visceral lesions of precisely the same nature, occurring in two patients who had received sulfathiazole on the surgical wards of two different Baltimore hospitals. Whether these focal visceral lesions are the result of hypersensitivity to sulfathiazole, or to a direct toxic action of the drug is not clear at present.

If the sulfonamides can cause the vascular lesions of periarteritis nodosa, as appears to be the case from the fresh lesions in our autopsy on the patient who had received sulfathiazole prophylactically and no serum, do they do so by a direct toxic action, or as a result of hypersensitivity? There is little doubt that hypersensitivity to the sulfonamides occurs. The "drug rash" may be frankly urticarial, and in its other forms may be quite like the non-urticarial forms of the serum sickness rash. Furthermore, the sulfonamide rash characteristically appears after a latent period of some days following the first dose; but once it has appeared, the readministration of the drug after a drug-free lapse of time tends to cause a prompt reappearance of the rash. Lyons and Balberor (8) have recently assembled similar evidence relating to the hypersensitive nature of the febrile reactions caused by sulfathiazole. Schönholzer (9) and Davis (10) have shown that the sulfonamides can attach themselves to the plasma proteins, and it is altogether probable that, acting in this manner as haptens, they are able to sensitize the body specifically.[1] It is of considerable interest in this connection that French and Weller (17) have described the occurrence of interstitial myocarditis, characterized by an infiltration of mononuclear and polymorphonuclear leucocytes including eosinophils, in patients who had been treated with sulfonamides. Since a precisely similar myocarditis was observed by Clark and Kaplan (3) in their cases of serum sickness in the absence of sulfonamide therapy, and since

[1] Wedum (19) has recently sensitized guinea pigs anaphylactically with sulfapyridine linked to human serum.

a similar lesion has been produced in sensitized animals subjected to repeated intravenous injections of foreign protein (18), it seems evident that this type of lesion can result from anaphylactic sensitivity. This strongly suggests that the eosinophilic myocarditis observed by French and Weller, may have been the result of hypersensitivity to the sulfonamides. It will be recalled that the same type of myocarditis was found in three of the present cases (Nos. 17250, 17257, 17291). Evidence that the urticarial rash in at least two of these cases (17257 and 17291) was due to serum sickness rather than to the sulfonamide therapy was presented above.

The possibility that periarteritis nodosa may be a result of hypersensitivity was suggested long ago by Gruber (11) and others. The presence of eosinophils in the lesion is, itself, suggestive. A number of writers have reported cases of periarteritis nodosa associated with bronchial asthma. Rackemann and Greene (14) reported eight cases of their own in which the two conditions were present, and found among 229 cases of periarteritis nodosa in the literature 19 in which it was mentioned that the patients had had asthma. From the reverse approach, it is interesting that among 29 cases of asthma collected by Kallós and Kallós-Deffner (15) in which death occurred during an asthmatic attack, and in which histological studies were made, an examination of the cited reports shows that there was one instance of frank periarteritis nodosa, and one case, described in 1892, with "marked" vascular lesions that were designated as "panarteritis obliterans" without further description.

The evidence provided by the cases described in the present paper, and by the two further cases in the literature in which lesions of periarteritis nodosa developed in patients with serum sickness, together with the experimental evidence that vascular lesions of this general type can occur in hypersensitive reactions to foreign protein, strongly indicates that periarteritis nodosa is a manifestation of anaphylactic hypersensitivity, and that widely different sensitizing antigens can be responsible for the development of the vascular lesions in different patients. It is not improbable that bacterial antigens may be concerned in some cases. If so, the present evidence indicates that the anaphylactic, rather than the tuberculin type of hypersensitivity (4) is implicated. While it may be that lesions of this type can also be

caused by the direct action of toxic agents without the intervention of hypersensitivity, the existing evidence relating these lesions to hypersensitivity is sufficient to warrant the most careful search for an inciting antigen in cases of periarteritis nodosa that are diagnosed clinically; for if the patient were freed from contact with the antigen in such instances, there is little doubt that the progress of the disease would be terminated. The literature contains cases of completely healed periarteritis nodosa, and a case showing only completely healed lesions has recently come to autopsy in this department.

SUMMARY

Vascular lesions characteristic of periarteritis nodosa have been found (1) in the viscera of five patients who, shortly before death, had had hypersensitive reactions following therapeutic injections of foreign serum. Four of these patients had received sulfonamides, but in at least two of those cases the evidence indicates that the hypersensitive reaction was serum sickness and not drug hypersensitivity. The fifth patient had serum sickness in the absence of sulfonamide therapy; (2) in a biopsy of muscle from a patient who had a hypersensitive reaction following foreign serum and sulfonamide therapy; (3) in the viscera of a patient who had received prophylactic sulfonamide therapy against aspiration pneumonia.

None of these patients had had any symptoms suggestive of periarteritis nodosa prior to their terminal acute illness for which the serum or sulfonamide was administered, and the vascular lesions were fresh.

These cases, together with other pertinent evidence discussed in the body of this paper, indicate that vascular lesions of this type can be a manifestation of the anaphylactic type of hypersensitivity, and suggest the importance of a search for the inciting antigen in cases of periarteritis nodosa that come under clinical observation.

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EXPLANATION OF ILLUSTRATIONS

FIGS. 1, 2 AND 3. Arteries in testis of Case 17628. The walls of the arteries in Figs. 1 and 2 are completely necrotic. The lumen in Fig. 2 is largely occluded. In Fig. 3 only a segment of the arterial wall is necrotic.

FIG. 4. Artery in ovary of Case 17291. The greater part of the circumference of the vessel is necrotic. There are many eosinophiles among the cells about the artery.

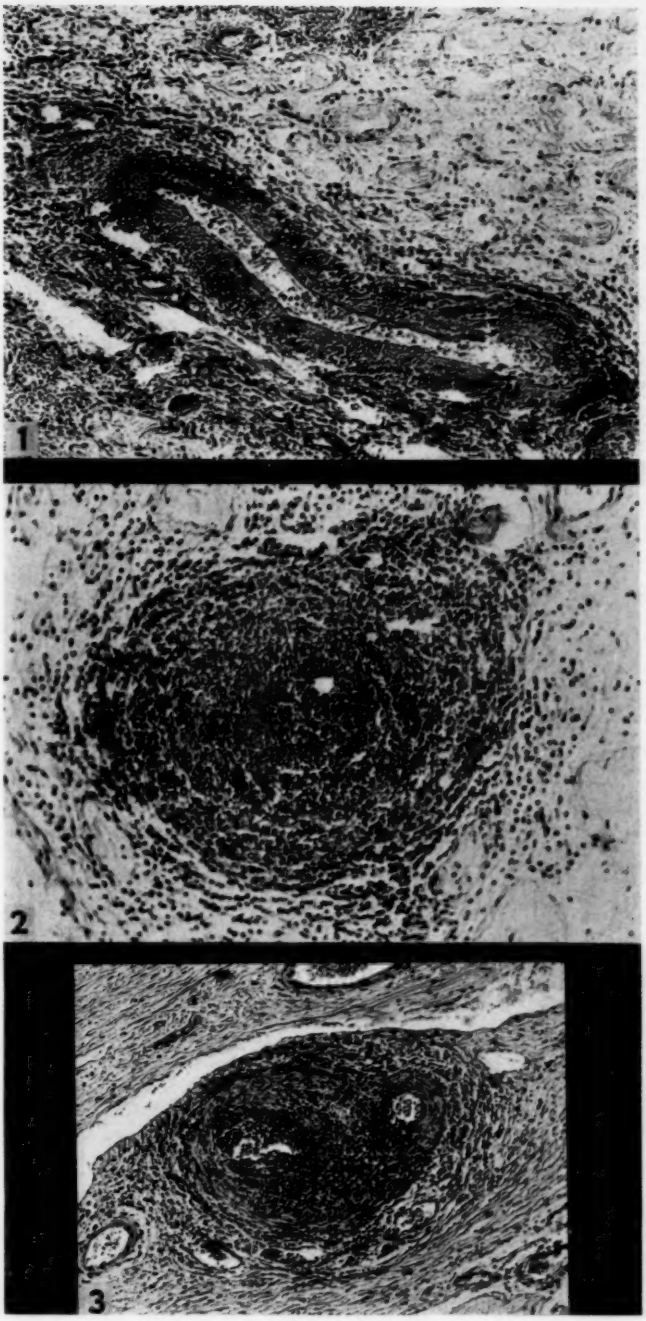
FIG. 5. Artery in renal pelvis in Case 17250. The lesion is very fresh. The necrotic wall of the artery shows fibrinoid alteration. There are many eosinophiles about the arteries in this case.

FIG. 6. Artery in meninges in Case 14664. There is necrosis and hyalinization of the wall.

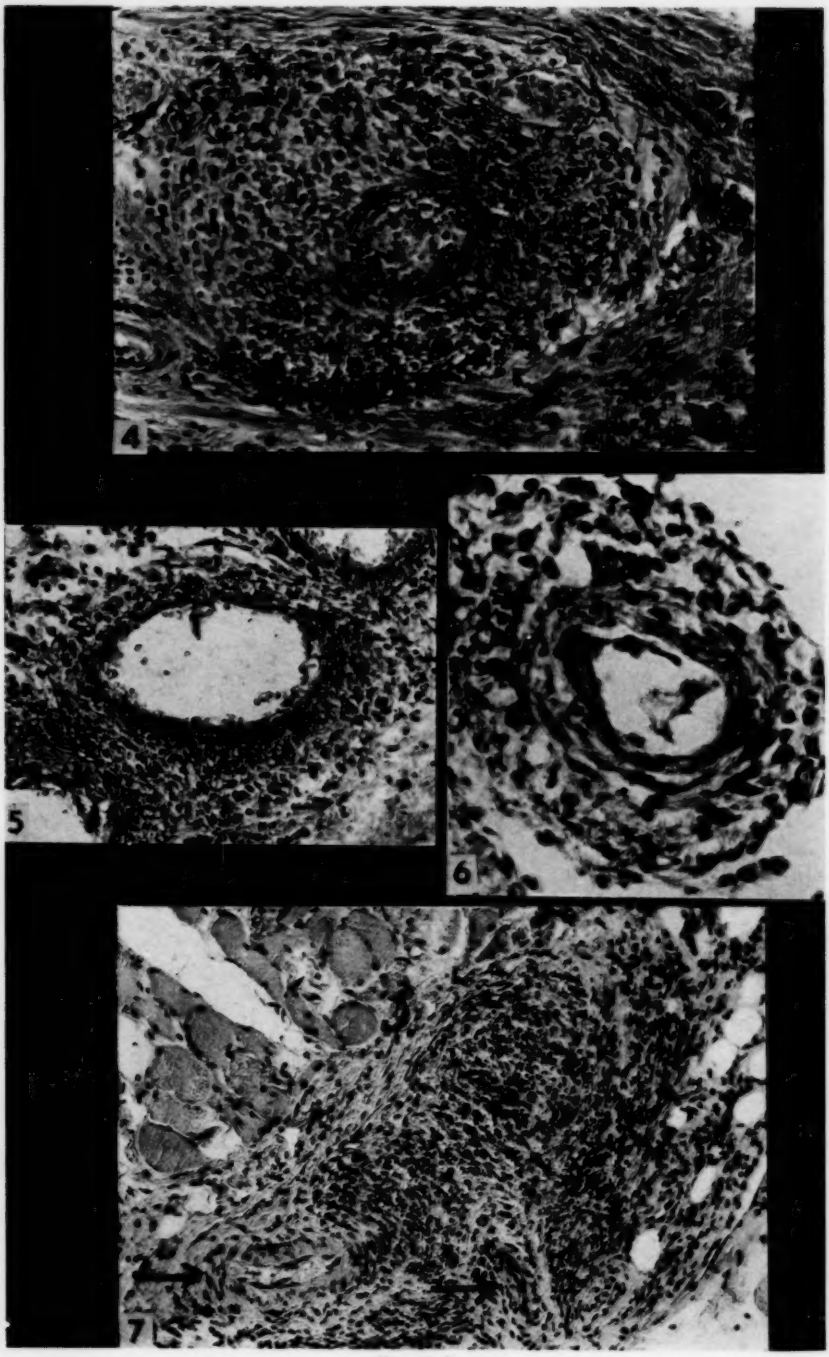
FIG. 7. Biopsy of biceps muscle of Case S. P. 81125. The arrows point to two arteries.

FIGS. 8, 9 AND 10. Arteries in the liver of Case 17257. The patient lived 3 weeks after the onset of serum sickness, and the lesions show more fibroblasts than in the cases of shorter duration.

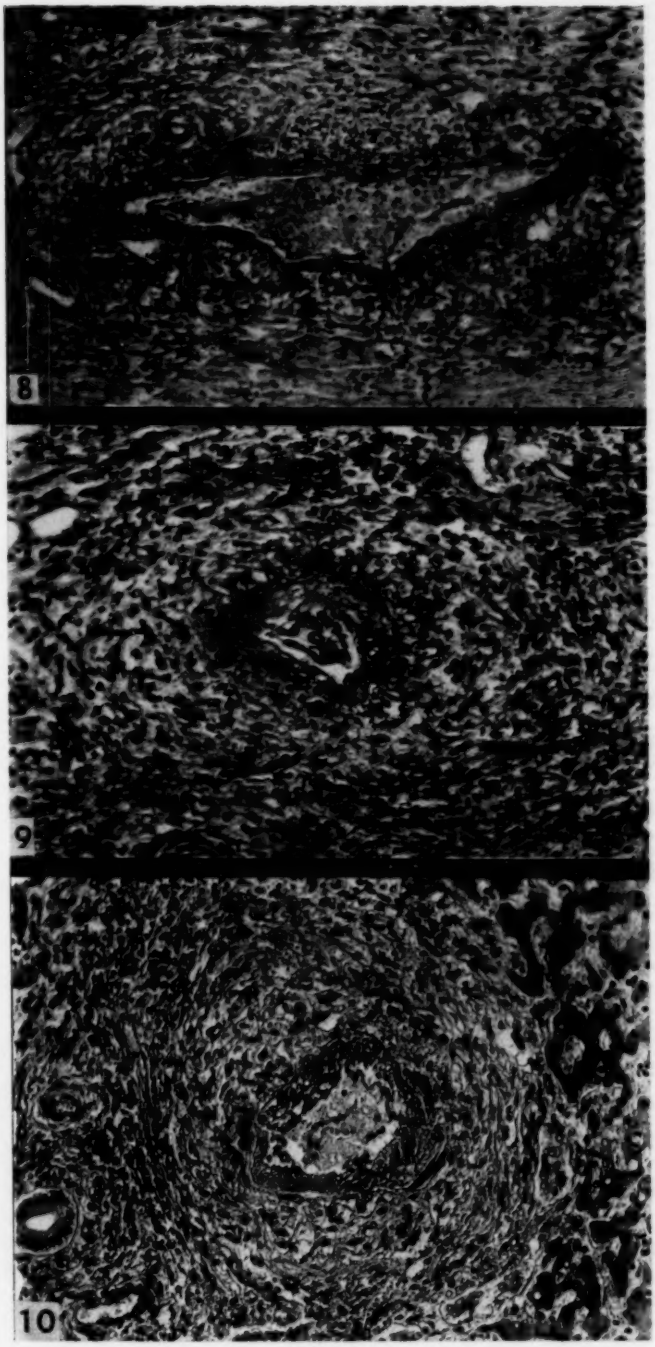
FIGS. 11, 12 AND 13. Arteries from Case 17529. Fig. 11 shows a small renal artery, with necrosis of part of the circumference. Fig. 12 is an artery in the tissue near a cervical lymph node; Fig. 13 is an artery within the node. The walls of both of these latter vessels are completely necrotic. That of Fig. 13 is hyalinized. There is marked perivascular infiltration with inflammatory cells.



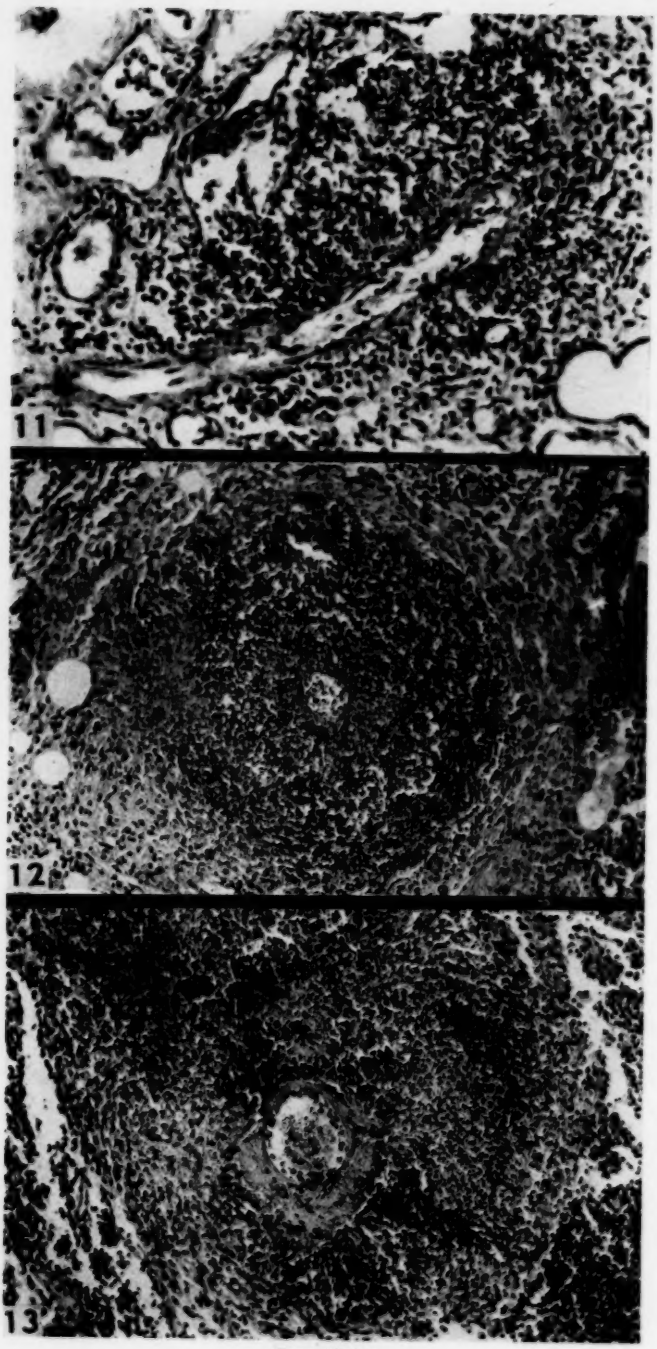
FIGS. 1–3



FIGS. 4–7



FIGS. 8–10



FIGS. 11–13

A STUDY OF THIAMINE DEFICIENCY IN SWINE

Together with a Comparison of Methods of Assay[1]

MAXWELL M. WINTROBE, HAROLD J. STEIN,[2] MITCHELL H. MILLER,[3] RICHARD H. FOLLIS, JR., VICTOR NAJJAR AND STEWART HUMPHREYS

From the Departments of Medicine, Pathology and Pediatrics, Johns Hopkins University

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The accepted concept of the effects of thiamine deficiency depends largely on animal experiments and, in man, on clinical observations in which the possible existence of deficiency of other substances at the same time cannot be excluded with certainty. Experiments in animals have been carried out chiefly by feeding yeast which was autoclaved or was otherwise treated with the object of destroying thiamine. It has not been shown conclusively that by such measures only this vitamin is affected. As to clinical observations, the assumption that in beriberi only thiamine deficiency occurs, implies on the part of the victim a knowledge of the distribution of vitamins in foods and an ability to select them which would be exceptional, to say the least.

In answer to the above criticism it has been pointed out that improvement or cure of symptoms has occurred following thiamine administration alone. Examination of the details of the evidence which has been presented, reveals that adequately controlled conditions have rarely been produced. Studies in man have been particularly defective in this respect (1). Certain experimental studies, it is true, have been carried out with meticulous care but it may be asked whether observations in pigeons, for example (2), can be accepted as necessarily applicable to man (3). Further evidence in other animals is needed.

The observation that young pigs can be raised satisfactorily on a simple diet poor in the "B" vitamins and supplemented only with pure

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[2] Fleischmann Fellow in Medicine.

[3] Upjohn Fellow in Medicine.

crystalline vitamins of this group (4), has made it possible to produce a deficiency of thiamine by a method which does not require autoclaving in order to remove this vitamin from the diet. By adding none or only inadequate quantities of thiamine to the mixture of crystalline vitamins, acute and relatively chronic states of thiamine deficiency have been produced.

These experiments were carried out concurrently with other studies in which deficiencies of pyridoxine, pantothenic acid and other factors were produced (4), while still other animals received thiamine, riboflavin, nicotinic acid pyridoxine, choline and pantothenic acid and grew very satisfactorily. In the animals given little or no thiamine, as well as in certain other pigs, the excretion of thiamine in the urine and the pyruvic acid content of the blood were measured. Consequently, the observations to be presented reveal not only the clinical picture of thiamine deficiency in the pig and the pathological changes which occur, but afford an opportunity to examine the value of these assay procedures as an index of thiamine deficiency.

EXPERIMENTAL PROCEDURE

Details concerning the management of the animals have been given elsewhere (5, 6). Pigs about 3 weeks of age are fed a basal diet consisting of Sheffield "New Process" crude casein, 26.1%; sucrose, 57.7%; lard, 11.0%; and a special salt mixture, 5.2%. One "unit" (36.4 gm.) furnishing about 152 calories, or a fraction thereof, is given per kilogram body weight per day. This is supplemented with 0.5 gm. cod liver oil and 3.0 gm. brewers' yeast (Mead Johnson) per kg. body weight daily. To produce a deficiency of one of the "B" vitamins, the yeast is withdrawn, usually over the course of 2 weeks, and it is replaced with pure, crystalline vitamins of the B group other than the one which it is proposed to omit. In our animals good growth and normal development have been observed when thiamine hydrochloride (0.51 to 0.13 mg. per kg. body weight daily), riboflavin (0.12 mg.), nicotinic acid (1.20 mg.), pyridoxine hydrochloride (0.20 mg.), choline chloride (10.0 mg.) and calcium pantothenate (0.50 mg.) were furnished instead of yeast.

This report deals mainly with observations on three experimental groups of three animals each (table 1). Group 1 ("thiamine omitted") were fed no yeast after they were 88 to 90 days of age. Instead riboflavin, nicotinic acid, pyridoxine, choline and pantothenic acid were given in the doses indicated above. No thiamine was given until pronounced symptoms of deficiency developed, as described below and as indicated in figure 2. In group 2 ("low thiamine") yeast was withdrawn sooner (69 to 77 days of age) but a very small quantity of thiamine (10 μg. per kg. body weight per day) was given together with the B vitamins

listed above. The animals in group 3, litter mates of group 1, were fed 1.5 gm. desiccated whole liver per kg. body weight daily from the age of 88 to 90 days, when yeast had been completely withdrawn. The liver was the same as that used in earlier experiments (6) and was known to be rich in the B vitamins with the exception of thiamine. In previous observations it had been found that when thiamine was given in addition to this amount of desiccated liver, excellent growth and development occurred (6). The amount fed may have furnished as much as 40 μg. thiamine per kg. body weight. In the course of time the quantity of thiamine in the liver probably decreased.

There were 8 control animals (table 1). These were litter mates of the animals in which thiamine deficiency was produced and served as controls also for pigs in which other types of deficiency were produced (4). In five of the control animals, chemical assays were conducted at the same time and in the same manner as in the deficient pigs. Some of the control animals were given 0.51 mg. thiamine per kg. body weight daily at first, but this was followed by such great excretion of thiamine in the urine that the dose was reduced to 0.13 mg. The other B vitamins were given to all the control animals in crystalline form and in the doses already indicated.

In addition to the observations on these pigs, the results of chemical assays on four thiamine deficient and three control animals forming part of another experiment have been added in order that an attempt might be made to evaluate certain chemical procedures which have been proposed for the assay of thiamine deficiency (see table 2 and figure 6).

Daily observations of the condition of the animals were made and they were weighed weekly. The animals were housed in pens equipped with wire screens to minimize coprophagy. Metabolism cages were used when total urine specimens were collected. These collections were made for two day periods every two weeks.

"B" vitamin supplements were given every other day, a two day dose being furnished at one time. In order to eliminate errors during the period urinary thiamine excretion was being measured, which might arise from incomplete or delayed absorption from the intestinal tract following oral administration, the vitamin supplements were injected intravenously on the first day of the collection period. The remaining doses in the two week period, however, were given by capsule, orally. An exception was choline chloride, which was furnished separately in aqueous solution and was always given by mouth.

Thiamine excretion in the urine was measured by the thiochrome method, as described by Najjar and Holt (7). As already stated, there were two 24 hour collection periods every two weeks, the first beginning immediately after the vitamin supplement was injected intravenously, and the second extending from 25 until 48 hours after the injection. All urine specimens were collected under toluol and acetic acid was added to make a concentration of 2 per cent.

The pyruvic acid content of the blood was measured by the method of Bueding and Wortis (8). Blood samples were obtained from the jugular vein. The animals usually struggled during the procedure and had to be held by two assistants.

Their muscular activity probably accounted for the relatively high values for pyruvic acid obtained even in normal animals. Towards the end of these experiments the modification in the pyruvic acid method suggested by Klein (9) for the purpose of eliminating aceto-acetic acid from the sample was incorporated. There was good correlation between the two procedures.

In addition to the studies already mentioned, morphological examinations of the blood were made at regular intervals and gastric analyses were performed from time to time.

RESULTS

The experimental data are presented in the accompanying tables and figures.

Symptoms

The outstanding symptoms of thiamine deficiency were failure of appetite, vomiting and sudden death. Poor growth was a less striking sign (figure 1). In the animals which died early no conspicuous reduction in growth rate occurred. In the animals maintained for longer periods by the administration of subminimal doses of thiamine, weight gain became impaired but the development of skeletal structure proceeded. At no time was the gait of the animals abnormal. In contrast to the pigs deficient in thiamine, vomiting never occurred in the control animals, the appetite scarcely ever failed to be anything but excellent and growth was consistently good. All but one of the controls were finally sacrificed and were found normal at autopsy. The only control animal which was not deliberately sacrificed died as the result of bleeding ulcers of the stomach and colon.

In group 1 (table 1 and figure 2), vomiting and anorexia developed 74 days after yeast had been withdrawn. The pyruvic acid content of the blood was high (5.85 to 6.84 mg. per 100 ml.) in all 3 animals at this time. One of these pigs (6-67) died suddenly 9 days later. The excretion of thiamine in the urine, in contrast to that found in the urine of control animals (figure 3), had been found low since it was first measured, 19 days after depletion of thiamine had commenced. On the 19th day of the experiment, furthermore, a test dose of 50 μg. thiamine per kg. body weight was followed by practically no excretion of thiamine in the pig which died and of reduced amounts in one of the other two (table 2).

The death of the two experimental mates of pig 6-67 was probably

prevented by the administration intravenously of 50 mg. thiamine in each. The pyruvic acid content of the blood dropped abruptly, but the urinary excretion of thiamine remained essentially unchanged. Since we were interested in producing "chronic" thiamine deficiency such as may be seen in man, the daily oral administration of small amounts of thiamine (8 to 35 μg. per kg. body weight) was commenced

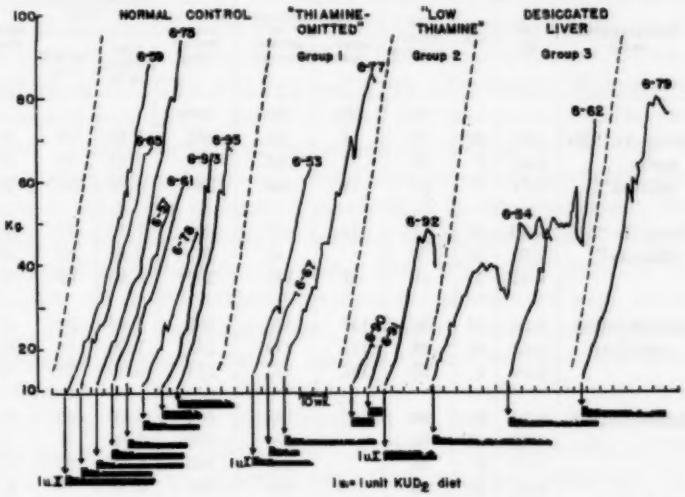

FIG. 1. GROWTH CURVES AND FOOD CONSUMPTION OF ANIMALS IN THE DIFFERENT EXPERIMENTAL GROUPS

All curves begin at the time the yeast supplement was omitted from the basal diet.

The broken line appearing at intervals represents the growth of pigs given a mixed diet at the Beltsville Research Center and fed and handled according to record of performance procedure.

33 days following the single injection of 50 mg. That a state of deficiency was maintained in these animals is indicated by the recurrence of anorexia and vomiting from time to time, by the elevated pyruvic acid content of the blood and by the low urinary excretion of thiamine.

Pig 6-53 died after a deficiency period of 156 days. One hour before death the animal appeared short of breath, its voice was weak and it was reluctant to move although it was able to walk. The heart beats

were about 110 per minute; the sounds were of good quality and no murmurs were heard. No rales were found in the lungs although the animal exhibited marked dyspnea at this time. Fifteen minutes before

TABLE 1

Tabulated data concerning experimental groups

EXPERIMENTAL GROUP	PIG NO.	SEX	AGE YEAST OMITTED	ONSET OF SYMPTOMS*	DURATION OF EXPERIMENT†	AVERAGE DAILY WEIGHT GAIN‡	BLOOD AT TIME OF DEATH		MODE OF DEATH§
							RBC millions × 10^6	MCV¶ c. μ.	
			days	*days*	*days*	*grams*			
Group 1: "thiamine omitted"‖	6-53	M	90	72	156	352	5.80	55	D
	6-67	F	88	74	83	212	7.25	54	D
	6-77	F	88	74	246	283	4.90	67	D
Group 2: "low thiamine"	6-90	M	77	41	57	200	7.35	57	D
	6-91	M	70		37	281	6.55	63	D
	6-92	M	69	41	141	202	3.68	60	K
Group 3: "desiccated liver"	6-54	M	90	117	320	105	6.47	55	D
	6-62	M	88	115	239	257	7.90	54	K
	6-79	F	88	135	230	278	6.80	51	K
Normal control	6-59	M	90		233	339	7.70	66	K
	6-65	M	88		194	298	5.05	71	D
	6-75	F	88		240	342	6.60	65	K
	6-57	M	90		146	286	6.55	59	K
	6-61	M	88		148	307	6.60	62	K
	6-78	F	88		149	245	5.65	72	K
	6-93	M	77		143	387	6.85	64	K
	6-95	M	70		146	391	6.05	65	K

* "Onset of symptoms" refers to the onset of anorexia and vomiting. The number of days is calculated from the time no more yeast was given and only crystalline "B" vitamins were fed.

† The experiment is considered as having commenced at the time when no more yeast was given.

‡ During the experimental period.

§ D = died; K = sacrificed.

¶ MCV = mean corpuscular volume.

‖ Following the death of pig 6-67 as the result of acute thiamine deficiency pigs 6-53 and 6-77 were given small amounts of thiamine from time to time as shown in figure 2.

death the animal was cyanotic. It staggered, fell, struggled stuporously to its feet, fell again on its side and lost consciousness. Just prior to death several convulsive movements were observed.

After an experimental period of 246 days' duration, pig 6-77 was exercised. The blood level of pyruvic acid was 5.6 mg. at this time. The animal was reluctant to move about and, soon after he was exercised, dyspnea became evident and audible. Soon cyanosis was noted and in time this became pronounced. It was especially noticeable in the ears, the snout and the lips. As time went on it became more and more difficult for the animal to move about and finally, after about 45 minutes, it collapsed following a brief series of jerky movements in the hind quarters. When the animal recovered, it was exercised again. This was followed again by collapse. Convulsive movements and finally death occurred one hour after exercise was started. Cyanosis and dyspnea had become extreme by this time.

In group 2 ("low thiamine," table 1), the period of observation was shorter. Yeast was withdrawn sooner than in the pigs already described but a small quantity of thiamine (10 μg. per kg. body weight) was furnished in the vitamin capsule from the beginning of the experiment. It is also noteworthy, since our animals are kept out of doors, that this experiment was conducted during cold weather (September to March) rather than in warmer weather (March to October) as was the case in group 1. Pig 6-91 died suddenly without premonitory symptoms after receiving no yeast and only 10 μg. thiamine per kg. for 37 days. Pig 6-90 died 20 days later after a brief period marked by no symptom other than occasional vomiting. By increasing the dose of thiamine to 30 μg. per kg. and then varying the dose between this level and 20 μg., pig 6-92 was maintained for a much longer period than its mates. It had to be sacrificed finally because of a severe infection in one leg.

In the pigs fed desiccated whole liver as the only source of the "B" vitamins (group 3, table 1 and figure 4), the symptoms and chemical changes were similar in every respect to those observed in the animals given only crystalline "B" vitamins. It was possible to maintain the period of deficiency for a long time in these animals, in part, no doubt, on account of the thiamine furnished by the liver. At times additional quantities of thiamine were given (figure 4). Pigs 6-62 and 6-79 were sacrificed after experimental periods of 239 and 230 days, respectively, and pig 6-54 died suddenly 320 days after yeast was replaced by desiccated liver, having received no added thiamine during its last 44 days of life other than that available in the liver.

Gastric analyses were carried out from time to time, histamine being used as the secretory stimulant. In the same animal great variations were noted, achlorhydria at one time, significant amounts of hydrochloric acid at other times. No correlation with the evidences of thiamine deficiency was noted.

Data for red cell count and mean corpuscular volume at the time of death in each animal are shown in table 1. Anemia of moderate or even severe degree was observed in several pigs but this seemed more closely related to the presence of infections than to thiamine deficiency.

Chemical studies

The results of measurements of the urinary thiamine excretion are shown in figures 2 and 3. It may be pointed out that in the control animals a significant though variable proportion (4.8 to 39.6%) of the injected dose of thiamine was excreted on the day of injection and a much smaller quantity on the following day. The excretion on the second day was usually greater than in the pigs given no thiamine, although this was not always the case.

It has been pointed out already that in the "thiamine omitted" group the urinary thiamine excretion was low as early as 19 days after depletion of thiamine had commenced (figure 2), 41 days before symptoms appeared in any of the animals. Furthermore, in two of these animals (6-53, 6-67) even at this time little excretion of thiamine followed the injection of a test dose of thiamine (table 2).

The pyruvic acid content of the blood was measured many times in the control animals and also in a number of pigs given thiamine but lacking some other vitamin (table 3). It should be pointed out that none of the samples of blood could be collected under basal conditions. Nevertheless the results were rather uniform. Only 3 values in the control animals were greater than 4 mg. These higher values, furthermore, were obtained early in this study, when the pigs had not yet become accustomed to venepuncture and struggled more when this was done than they did later. The value 3.50 mg. per 100 ml. was chosen, on the basis of the observations presented in figure 5 and summarized in table 3, as the upper limit of normal in the pig under the conditions of our experiment. It will be noted that this value was rarely exceeded in normal animals or in those given thiamine but lack-

ing some other vitamin (figure 3 and table 3) but was usually elevated above this level in the pigs deficient in thiamine (figure 2) except after thiamine was given.

Increases in the blood pyruvic acid occurred as thiamine deficiency progressed and the magnitude of the increases was usually closely related to the severity and seriousness of the symptoms. A prompt fall in the blood level after administration of thiamine and a return to high values when it was not given, were observed regularly and many times (figures 2 and 4). An additional factor which was sometimes associated with a fall in the blood pyruvic acid was the development of anorexia. This was noted several times (figures 2 and 4).

In order to secure further data which might be useful in evaluating the various chemical assay procedures, urinary thiamine excretion and blood pyruvic acid before and after the administration of 50 μg. thiamine per kg. body weight, were measured in 7 pigs not forming part of the main experiment here described. All of these pigs had received our standard basal diet supplemented with crystalline vitamins, but four (7-08, 7-09, 7-40, 7-41) had been given suboptimal amounts of thiamine whereas the other three (7-27, 7-32, 7-36) had received adequate quantities (260 μg. per kg. body weight daily). Three of the animals receiving suboptimal amounts had been given only 20 μg. thiamine per kg. body weight daily for 50 to 72 days prior to the time the measurements recorded in table 2 were made. Pig 7-40, at the time of the assay, was receiving autoclaved yeast as the sole source of the vitamin B complex. It is assumed that this furnished no thiamine. Complete data for these pigs and for 6 pigs already described in the preceding pages are given in table 2.

With the object of attempting to evaluate the chemical assays, the data for all the animals have been arranged in table 2 in the order of decreasing thiamine deficiency as judged by thiamine intake, food consumption and general condition and without regard to the results of the chemical assays. Nevertheless, corresponding to the degree of deficiency as judged by clinical criteria and estimates of thiamine intake, the lowest values for thiamine excretion and the highest values for blood pyruvic acid are to be found at the top of the table and the greatest urinary thiamine excretion and the lowest blood pyruvic acid values are found at the bottom.

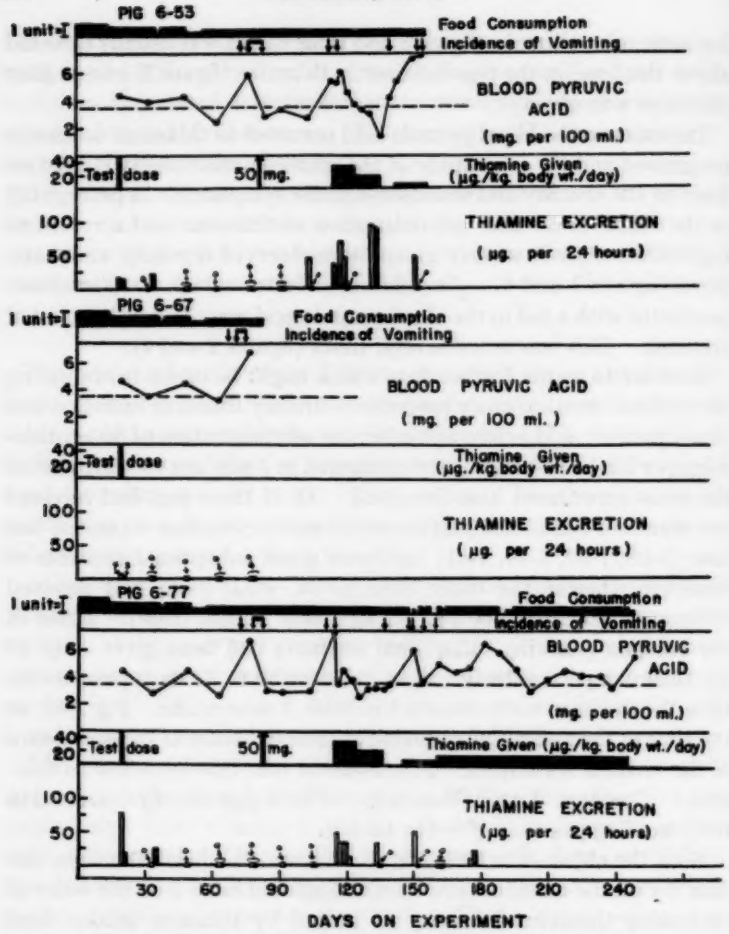

FIG. 2. CONTENT OF PYRUVIC ACID IN THE BLOOD, URINARY THIAMINE EXCRETION, FOOD CONSUMPTION AND INCIDENCE OF VOMITING IN THREE PIGS GIVEN SYNTHETIC VITAMINS WITH THE EXCEPTION OF THIAMINE AFTER THE AGE OF THREE MONTHS (GROUP 1, "THIAMINE-OMITTED")

As shown in the chart, thiamine was given from time to time only as a "test dose" (50 μg. per kg. body weight), as a single temporary curative dose of 50 mg. intravenously (pigs 6-53 and 6-77), and in sub-minimal doses by mouth. The object of the latter was to produce a state of "chronic" thiamine deficiency.

The interrupted line represents the upper limit of blood pyruvic acid in animals not deficient in thiamine.

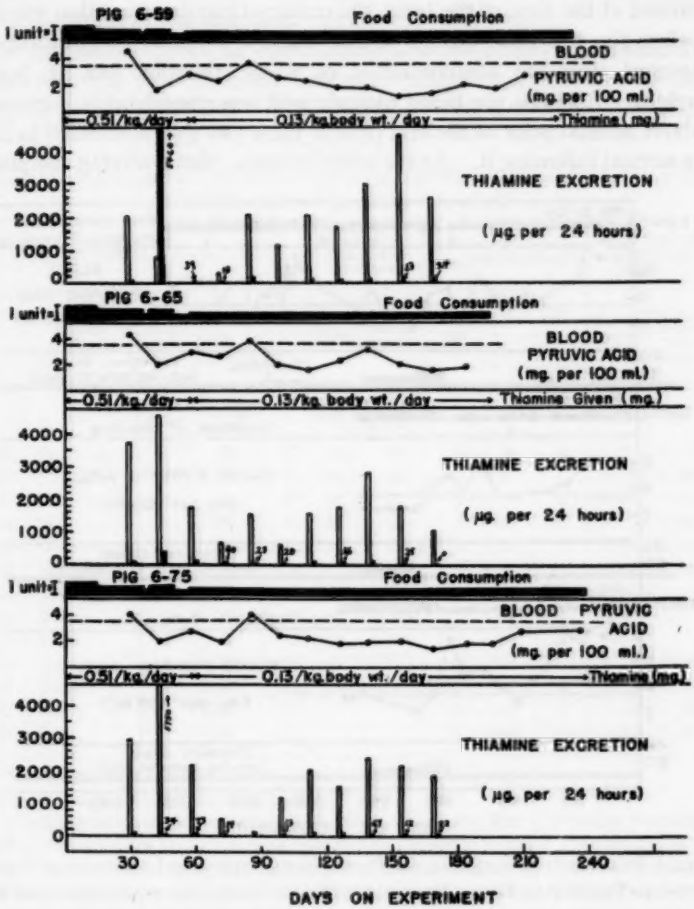

FIG. 3. CONTENT OF PYRUVIC ACID IN THE BLOOD, URINARY THIAMINE EXCRETION AND FOOD CONSUMPTION IN THREE CONTROL PIGS GIVEN SYNTHETIC VITAMINS INCLUDING THIAMINE

Thiamine was given every other day. Legends are similar to those in figure 2, but note that the scale for urinary thiamine excretion *differs* from that in figure 2. The open columns indicate the excretion in the 24 hours following the injection of thiamine, the solid columns the excretion in the succeeding 24 hour period.

In the two pigs in which actual symptoms of thiamine deficiency existed at the time of the tests, the urinary thiamine excretion was nil before the test dose was given and was the same or insignificant in amount after the administration of 50 μg. thiamine per kg. body weight. Likewise the blood pyruvic acid was considerably increased above normal prior to the test dose in these two pigs and failed to fall to normal following it. At the other extreme, pigs receiving adequate

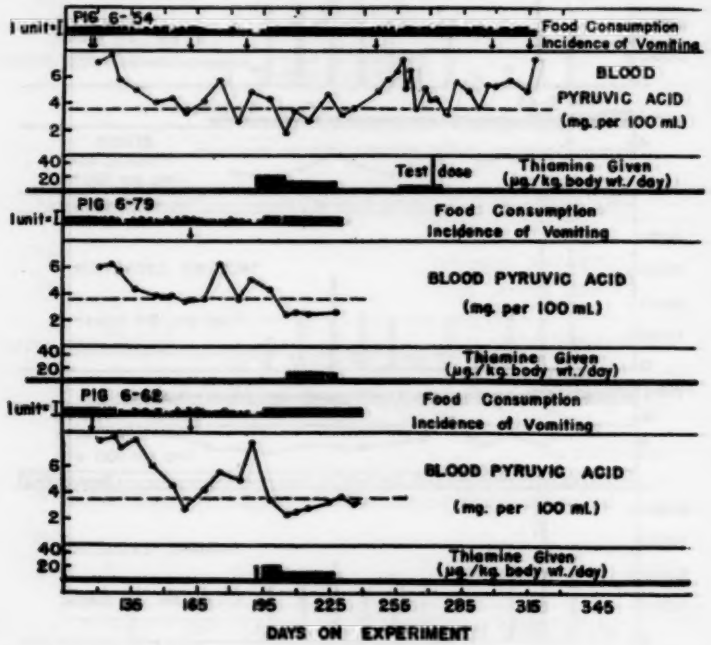

FIG. 4. PYRUVIC ACID IN THE BLOOD, FOOD CONSUMPTION AND INCIDENCE OF VOMITING IN THREE PIGS GIVEN DESICCATED WHOLE LIVER AND NO CRYSTALLINE B VITAMINS EXCEPT AS INDICATED UNDER THE HEADING "THIAMINE GIVEN"

amounts of thiamine were found to excrete thiamine before and in larger amounts after the test dose. In the same animals the blood pyruvic acid was, with one exception (6-95) found to be normal even before the administration of a test dose of thiamine. In pig 6-95 the

blood pyruvic acid fell below the upper limit of normal following the administration of the test dose.

In the animals given an intermediate position in table 2 reasonably good correlation between the estimated thiamine intake and the results of the chemical assays was also found. Pig 6-92 has been placed

TABLE 2

Thiamine excretion and blood pyruvic acid before and after intravenous administration of test dose of thiamine (50 μg. per kg. body weight)

PIG NO.	DESCRIPTION AT TIME OF ASSAY				THIAMINE INTAKE PRECEDING PERIODS		THIAMINE EXCRETION, (μG. PER 24 HOURS)			BLOOD PYRUVIC ACID (MG. PER 100 ML.)	
	General condition	Food intake	Thiamine intake		Time	Average amount	Before test dose	Following test dose		Before test dose	24 hrs. after test dose
			Amount	No. of days				First 24 hrs.	Second 24 hrs.		
		kilo units	*μg./kg. body wt. per day*		*days*	*μg./kg. body wt./day*					
7-40	Vomiting	¼	0	27	55	50	0	0	0	6.51	3.63
6-92	Vomiting	1	30	19	79	14	0	10		8.20	7.60
7-41	Mod. good	½	20	50	41	13	6	7	0	4.72	3.10
7-08	Mod. good	½	20	72	42	13	5	28	12	4.18	2.48
7-09	Good	½	20	54	38	13	18	26	2	3.44	2.56
6-54	Inactive	½	45*	276			0	7		4.34	4.34
6-67	Mod. good	¾	0	19	66	250†		4	3	4.90	
6-53	Good	¾	0	19	65	250†		17	14	4.42	
6-77	Good	¾	0	19	65	250†		76	16	4.44	
6-95	Good	1	130‡	96	47	250†	0	162		4.92	3.24
7-27	Good	¼	260	109			28	43	44	2.92	2.36
7-32	Fair§	½	260	116			20	42	40	1.44	1.14
7-36	Fair	1	260	117			21	52	24	1.56	1.30

* Of this amount all but 5 μg. was derived from desiccated whole liver.

† This was furnished in brewers' yeast.

‡ In this and in the three animals which follow, the regular dose of thiamine was withheld on the day prior to the thiamine tolerance test in order to furnish comparable data.

§ In pigs 7-32 and 7-36 pyridoxine and pantothenic acid deficiency had been produced but were under treatment at the time of the thiamine assay. These animals had always received adequate amounts of thiamine.

second on the list in spite of a greater thiamine intake than in some of the animals which follow it because the quantity of food it consumed was twice that of 7-41, 7-08 and 7-09.

A discrepancy between expected and observed results occurred in the case of pig 6-54 which, according to the results of the chemical

assays, should follow or immediately precede pig 7-41 in table 2. According to thiamine intake it was placed lower in the table. It is of interest, however, that this pig received most of its thiamine in desiccated liver and it is the only animal in which thiamine intake was not known exactly. It may be that our estimate of thiamine intake rather than the chemical assay was inaccurate. The failure of pig 6-95 to excrete any thiamine before the administration of a test dose is surprising in view of the quantity of thiamine the pig received and the large amount excreted following the test dose. In all the other instances in table 2 the correlation of known thiamine intake and the

TABLE 3

Pyruvic acid in the blood of pigs not deficient in thiamine

EXPERIMENTAL GROUP	NUMBER OF DETERMINATIONS	AVERAGE VALUE‡	RANGE OF VALUES
		mg. per 100 ml.	
Normal control	60	2.41	1.08–4.56
Nicotinic acid omitted*	24	2.12	1.07–3.53
Pyridoxine omitted*	7	2.29	1.66–3.14
Low protein†	6	2.18	1.90–2.54
Pyridoxine and choline omitted*	4	1.80	1.38–3.80
Miscellaneous	7	2.22	1.54–2.86
Total	108	2.37	1.07–4.56

* Detailed observations on animals in these groups are reported elsewhere (4).

† These animals were fed synthetic vitamins including thiamine. They differed from the normal controls in that the casein content of the diet was 10% instead of 26.1%.

‡ The Standard Deviation of these values is 0.72 mg., the Coefficient of Variation is 30.4%.

results of the chemical assays is good. It is noteworthy that of the three experimental mates, pigs 6-53, 6-67 and 6-77, pig 6-67 was the most deficient according to chemical assay and this animal was the first of the three to die of thiamine deficiency.

The effect of the administration of glucose upon the pyruvic acid content of the blood was studied in five thiamine deficient and in four normal pigs (figure 6). Glucose, 1.75 gm. per kg. body weight, was given in a 25% solution through a stomach tube. The blood pyruvic acid was measured before glucose administration and 1, 2 and 4 hours afterwards. In the normal animals the blood pyruvic acid was es-

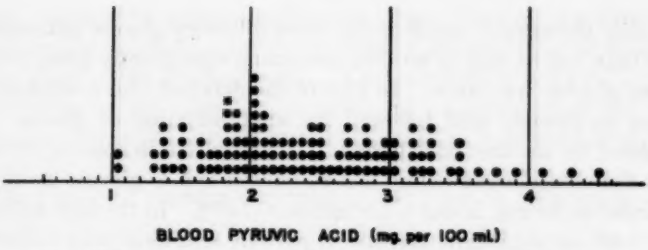

FIG. 5. PYRUVIC ACID IN THE BLOOD OF PIGS GIVEN THIAMINE IN ADEQUATE AMOUNTS

The samples of blood were not taken under basal conditions since the animals' muscular activity could not be restricted a great deal.

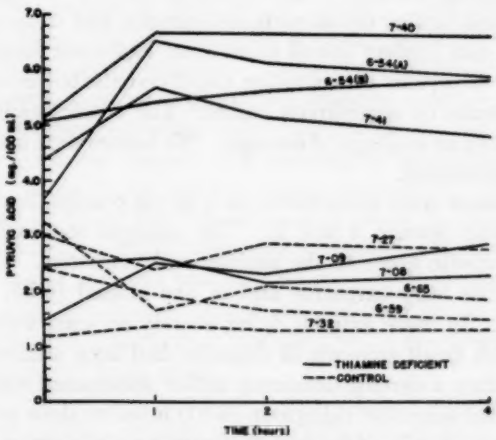

FIG. 6. BLOOD PYRUVIC ACID BEFORE AND AT INTERVALS AFTER THE ORAL ADMINISTRATION OF GLUCOSE (1.75 GM. PER KG. BODY WEIGHT) IN 5 THIAMINE DEFICIENT AND 4 CONTROL PIGS

Data concerning most of these animals will be found in table 2. These glucose-pyruvic tolerance tests were carried out 33 days following the thiamine excretion tests listed in table 2 in the case of pigs 7-09, 7-40 and 7-41, 14 days after these tests in pigs 7-08 and 7-27 and 8 days after the test in pig 7-32. They were done 142 days (A) and 8 days (B) *before* the thiamine excretion test in pig 6-54. The thiamine intake in the intervals was not changed.

Pigs 6-59 and 6-65 were control animals receiving 130 μg. thiamine per kg. body weight per day.

sentially the same or significantly lower following glucose administration than before, and at no time was found significantly greater than before glucose was given. In four of the deficient pigs a marked increase in pyruvic acid followed the administration of glucose and persisted for the four hour period. This occurred in spite of the fact that the values found before the administration of glucose would be regarded as normal in one of the animals (7-09). In the fifth deficient pig (7-08) no significant increase in pyruvic acid took place following the administration of glucose. This was not unexpected for the deficiency was only moderate in degree.

Pathological findings

Nervous system: Portions of the brachial, sciatic and vagus nerves, the cervical and lumbar dorsal roots and ganglia, and cross sections of the cervical and lumbar spinal cords were examined histologically. No evidence of myelin degeneration could be detected in any of the deficient animals by appropriate stains. The sensory ganglion cells likewise showed no evidence of damage. No lesions were found in any of the control animals.

Heart: Lesions were encountered in 5 of the 6 animals given little or no thiamine (groups 1 and 2). The changes consisted of focal necroses of muscle fibres in the auricles and ventricles. Such areas were sometimes very extensive and in one animal (6-53) could be seen grossly. In those animals dying acutely no scars were seen; in those to which small amounts of thiamine had been administered in order to produce a chronic deficiency rather widespread scarring was observed. One animal of this group (6-92) failed to show any lesions. This animal was sacrificed because of the presence of a severe infection in one leg, after having received larger amounts of thiamine than the other animals and at a time when the blood pyruvic acid had been normal for 41 days. In the pigs dying suddenly pulmonary edema was observed. No chronic passive congestion of the liver was found.

Similar changes in the heart consisting of fresh areas of necrosis and scars were found in 1 of the 3 animals receiving desiccated liver (table 1, group 3). It is noteworthy that this animal (6-54) lived longer than the other 2 animals and died suddenly. His mates were sacrificed at a time when the blood pyruvic acid had been normal for 45 and 30 days respectively (figure 4).

No lesions were encountered in the control animals nor have we noted similar changes in the myocardium of animals deficient in other components of the B complex (4). That inanition is not a factor has been shown by our failure to find lesions in 2 animals whose diet was adequate though the amount was restricted so as to produce very poor growth (5).

The histologic changes occurring in the heart in association with thiamine deficiency will be described in detail elsewhere (10).

DISCUSSION

The experiments described in this report appear to be the first studies of thiamine deficiency in which crystalline vitamins have been the chief source of the vitamin B complex. It may be noted that the casein fed our animals is described as "crude." This casein contains riboflavin (about 4 μg. per gram) but probably furnishes little or none of the other recognized vitamins. Certainly it carries little thiamine, since we have succeeded in producing thiamine deficiency; it also affords no substance protecting against sensory neuron degeneration for when it is fed in the absence of pyridoxine or pantothenic acid, degenerative changes in the nervous system develop (4). Furthermore, other than riboflavin the "crude" casein seems to furnish little or nothing required by swine other than is found in a purified casein, for when the latter was fed and supplements of thiamine, riboflavin, nicotinic acid, pyridoxine, choline and calcium pantothenate were given, growth and development took place which were practically equal to that of pigs fed crude casein (4).

Having found so highly refined a diet which is suitable for pigs, it has been possible to deprive them of thiamine without at the same time altering the quantities of other vitamins. This, we believe, is the most important feature of the studies we are reporting. Before crystalline "B" vitamins were available, experimental "thiamine" deficiency was produced by feeding a crude substance, rich in the vitamin B complex, which was first treated in such a way that "vitamin B_1" was destroyed. Autoclaving has been the method most commonly used for the destruction of the thermolabile portion of the vitamin B complex. When thiamine was discovered, it was assumed by many investigators that thiamine is the only thermolabile factor in the B complex and the terms B_1 and thiamine have been regarded as synony-

mous. It seems curious that in experiments designed to study thiamine deficiency it has not been deemed necessary to prove that a vitamin B complex-free diet, when supplemented with autoclaved yeast plus thiamine, will support normal growth and development for a long time. Thus, even in Swank's (2) careful study of "avian thiamine deficiency" the importance of such a control series seems to have been overlooked.

In this connection the studies of Van Etten, Ellis and Madsen (11) are of special interest. Their experiment, designed to study thiamine deficiency in swine, described the effects produced when a diet was fed in which liver and whey were the chief source of the vitamin B complex. When the diet was treated with sodium sulfite-sulfur dioxide and fed to pigs, the symptoms developing were quite similar to those we have described in this paper as resulting from thiamine deficiency. This is consistent with the assumption that by such treatment only thiamine is destroyed. When, however, the same diet was *autoclaved* and fed to the animals, convulsions and nerve degeneration developed which were compared by Van Etten and his co-workers to those we first described (12) and which we have recently ascribed to pyridoxine and pantothenic acid deficiency (4). It is noteworthy that these workers observed that the changes in the nervous system were not affected by the administration of ample amounts of thiamine.

In the light of these facts, it does not seem surprising to us that no neurological symptoms and no changes in the nervous system were observed in the pigs in which we caused thiamine deficiency, even in those in which "chronic" thiamine deficiency of long duration was produced. It is a fact that the requirements of different species of animals for vitamins differ a great deal and it is evident that findings in pigs cannot necessarily be transferred to other animals or to man. We submit, however, that nerve degeneration which has heretofore been attributed to thiamine deficiency may as readily have been caused by an unrecognized lack of some other vitamin. Until experiments are reported in which nerve degeneration has been produced as the result of the omission of thiamine from the diet while other "B" vitamins have been furnished in adequate amounts in crystalline form, it seems to us that the case for thiamine as an antineuritic vitamin must be regarded as unproved (3).

It also seems likely that some of the disagreement which is to be

found in the literature regarding the manifestations of "B_1" and "thiamine" deficiency can be attributed to different degrees of autoclaving and to other variations in the treatment of the diets fed, with consequent qualitative as well as quantitative variations in the vitamins affected by such treatment.

Since we have previously reviewed the literature concerning the effects of vitamin deficiencies on the nervous system (5) and Meiklejohn (1) as well as Swank (2) have considered the thiamine literature in this respect at some length, no further discussion of this subject is necessary here.

In all of our thiamine deficient animals in which death occurred, the terminal symptoms were those of acute, severe cardiac failure, or unexpected death occurred without such manifestations as dyspnea, cyanosis and weakness having been observed. No edema other than in the lungs was ever found. Bradycardia was usually present. The pathological changes will be described fully in another report (10) where the cardiac manifestations of thiamine deficiency will be more fully considered. It may be pointed out that inanition is not an important factor in the production of the symptoms of thiamine deficiency for some of the animals (e.g., 6-90, 6-91) ate well at all times.

It is difficult to delineate symptoms of "chronic" as compared with "acute" thiamine deficiency in the pig, unless the former can be regarded as a symptomless phase of thiamine depletion while in the latter the dramatic consequences of deficiency are manifest. In our experience, once vomiting or anorexia have appeared, symptoms of cardiac failure may develop soon and very suddenly, and unexpected death may occur.

Although pyruvic acid levels in the blood can be elevated by any means which will increase carbohydrate metabolism, the data presented here demonstrate definitely that the abnormal elevation of this compound in the blood of the deficient pigs was due to a lack of thiamine and that in the pig, as well as in other experimental animals (13, 14, 15) thiamine diphosphate (cocarboxylase) is needed for the oxidation of pyruvic acid. It must be emphasized, however, that the method employed for the measurement of pyruvic acid most probably does not differentiate other closely associated carbonyl compounds such as alpha-ketoglutaric acid but probably measures them together with pyruvic acid. Recent studies by Barron and coworkers (16)

have demonstrated that alpha-ketoglutaric acid is affected by thiamine in a manner similar to pyruvic acid. Thus, although a direct relation between "pyruvic acid" metabolism and thiamine has been demonstrated by the data of this communication and by other investigators, it might be more valid to refer to this relationship as an alteration in carbonyl compounds, primarily pyruvic acid, instead of *only* pyruvic acid.

We have found the blood "pyruvic acid" level to be a useful guide in the study of thiamine deficiency in the pig, a sharp rise presaging the onset of serious symptoms with possibly fatal consequences. The value of the blood pyruvic acid as a measure of thiamine deficiency was not very much increased by determining the quantity of pyruvic acid in the blood following the administration of a test dose of thiamine (table 2), since a drop occurred usually in thiamine deficient as well as in normal pigs. A "load test" such as that suggested by Bueding and his coworkers (17) in which the blood pyruvic acid was measured before and after the administration of glucose was more useful, for an increase in pyruvic acid occurred in all of the thiamine deficient pigs whereas no significant increase or an actual decrease was observed in the control animals. From the data we have obtained so far, it would not appear that much is gained by measuring the blood pyruvic acid two and four hours after the administration of glucose as well as one hour later (see figure 6). This subject is under study. For practical purposes the values before and one hour after glucose administration may be found to suffice. Since the blood pyruvic acid is subject to fluctuations with exercise and other factors, such a functional test has more merit than a single determination and yet remains relatively simple.

From our observations it would appear that the urinary thiamine excretion reflects somewhat irregularly the immediately preceding thiamine intake, but the thiamine excretion test of Najjar and Holt (7) is a useful measure of thiamine subnutrition, retention of thiamine following the administration of a test dose being definitely related to the degree of deficiency as judged by clinical criteria and estimates of thiamine intake.

SUMMARY

1. Thiamine deficiency has been produced in pigs given crystalline vitamins as their chief source of the vitamin B complex.

2. The chief symptoms observed were anorexia, vomiting, dyspnea, cyanosis and great weakness. The symptoms of cardiac failure appeared suddenly and unless thiamine was given resulted promptly in death. Unexpected death occurred in some instances without dyspnea and cyanosis having been observed. Impairment of growth was not a prominent sign in pigs dying early of acute thiamine deficiency.

3. In all the animals dying as the result of thiamine deficiency, focal necroses of the myocardium were observed.

4. No neurological symptoms were observed, nor were any degenerative changes in the nervous system found even in animals in which thiamine deficiency of long duration was produced.

5. It is suggested that nerve degeneration heretofore attributed to thiamine deficiency may be due to lack of other vitamins, deficiency of the latter having been produced by the process whereby it was expected that only thiamine would be destroyed.

6. In the experimental animals and in controls, urinary thiamine excretion, the excretion of thiamine following the administration of a test dose, and the content of pyruvic acid in the blood were measured and their value as measures of thiamine deficiency noted.

7. The observations suggest that the urinary thiamine excretion reflects only the immediately preceding thiamine intake; the thiamine tolerance test is a useful measure of thiamine deficiency states; the blood pyruvic acid, although influenced by factors in addition to thiamine and carbohydrate intake, was found to be correlated with the onset and severity of symptoms of thiamine deficiency. More useful than a single determination of blood pyruvic acid, however, was comparison of the value before and after the administration of a measured amount of glucose.

Vitamins used in these experiments were furnished by Merck and Company, Incorporated, and cod liver oil and yeast by Mead Johnson and Company.

Dr. Theodore L. Danforth, Jr. and Dr. Raul Alcayaga assisted in some of these studies and technical aid was given by Mr. Adolph Suksta, Mrs. Lottie Lowenstein, Mrs. Eleanor Collins, Miss Eva Hodgens, Miss Elaine Cohen, Miss Gertrude Merr and Mr. William Mock.

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OBSERVATIONS ON THE NATURE OF TETANY

The Effect of Adrenaline

A. M. HARVEY and J. L. LILIENTHAL, JR.

From the Department of Medicine, The Johns Hopkins Hospital and University

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The clinical manifestations of tetany which have received the most attention are those spasms of skeletal muscle which involve the hands and feet. There are, however, many observations on the concomitant forms of muscle spasm which involve structures innervated by the autonomic nervous system. Falta (1909, 1912) first demonstrated that the subcutaneous injection of small amounts of adrenaline in subjects with latent tetany evoked carpopedal spasm and extreme blanching of the skin, cooling of the extremities, and tachycardia of far greater degree than was produced by a similar amount of adrenaline in normal subjects.

During a study of the neurophysiological mechanisms underlying the development of tetany we have had the opportunity to observe muscle and vasomotor responses to the intra-arterial injection of adrenaline in three patients with hypocalcemic tetany, one patient who exhibited tetany after brief periods of hyperventilation, and one patient who developed tetany during periods of emotional tension. The observations reported here contribute toward an understanding of the rôles played by the vascular system and by hypocalcemia in the pathogenesis of tetany. The danger attendant upon the administration of adrenaline to patients subject to tetany is emphasized.

METHODS

Adrenaline, in a 1–1000 solution, was injected into the brachial artery through a subcutaneous bleb of procaine. Immediately before injection a sphygmomanometer cuff placed proximally about the arm was inflated suddenly to a pressure of 30 to 50 mm. of mercury. The pressure employed was determined before injection to be less than that required to elicit Trousseau's sign in the particular individual. In the normal subjects cuff pressures of 50 to 60 mm. were employed.

The cuff remained inflated for one to two minutes to obstruct the venous outflow from the arm and was then deflated. The patients had been warmed before injection by electric heating pads, blankets and by immersion of one leg in a water bath held at a temperature of 42 degrees to 45 degrees C. Generalized vasodilatation had occurred as evidence by flushing, sweating and skin temperatures of 35 degrees to 36 degrees C. Heating of the body was continued throughout the experiment. Observations were made of the injected extremity and comparison made with the control side with reference to color, development of tetany, and the volume of the radial arterial pulse as estimated by palpation. The skin temperature of the fingers and toes of the injected and control extremities was recorded by copper-constantin thermocouples balanced against a constant-temperature water bath through a galvanometer. Control injections of physiological saline and puncture of the brachial artery alone did not produce significant changes in skin temperature nor did they induce tetany in the normal subjects or in those with hypocalcemia.

MATERIAL AND RESULTS

Normal Subjects: When heating had produced generalized vasodilatation and sweating, 0.15 to 0.6 mg. of adrenaline was injected into the brachial artery of three normal subjects. This resulted in immediate blanching and cyanosis of the extremity, marked reduction in the volume of the radial pulse, cessation of sweating in the injected area and the subjective sensation of cold but no pain or paresthesiae. These results did not differ from those observed by Myerson, Loman, Rinkel, and Lesses (1938). The injection of adrenaline was followed immediately by an abrupt fall in the skin temperature of the injected extremity toward that of the environment. The extremity became progressively cooler for 10 to 15 minutes and then began to warm; within 20 to 45 minutes the skin temperature had returned to its preinjection level (Figure 1). During the period of warming, the volume of the radial pulse increased and as the flush reappeared, sweating returned. When the cuff was deflated one minute after the injection, the familiar general symptoms produced by adrenaline were noted. These were transient apprehension, heart consciousness, headache, tachycardia and slight facial pallor. In addition the skin

temperature of the other extremities fell 2 to 3 degrees C. but returned to their former level within 5 minutes. In the normal subjects there were no movements of the injected extremity.

Hypocalcemia: There were three patients in this group.

Patient I. W. (Unit #225434), an eighteen-year-old white housewife, developed outspoken symptoms of hyperthyroidism in the autumn of 1939 and underwent a subtotal thyroidectomy in May, 1941. On the third post-operative day she presented signs of manifest tetany and at this time the concentration of serum calcium was 7.0 mg. per cent and of phosphorus, 3.6 mg. per cent. Despite the oral administration of calcium chloride the serum calcium ranged below 7.0 mg.

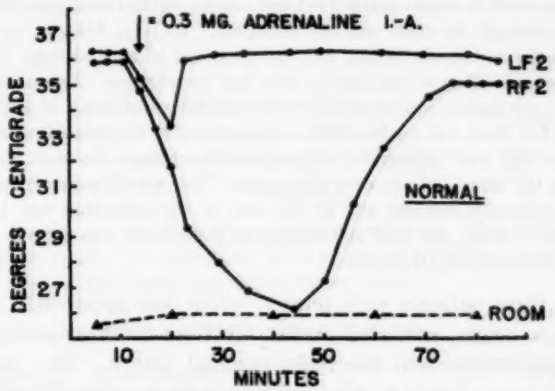

FIG. 1. Normal Subject. Skin temperatures of left (LF2) and right (RF2) second fingers. Adrenaline (0.3 mg.) injected into the right brachial artery at arrow.

per cent and the serum phosphorus above 5.5 mg. per cent; and there were recurring attacks of tetany, many of which were associated with episodes of anxiety and temper tantrums. When first tested with adrenaline the serum calcium was 7.6 mg. per cent and the phosphorus was 5.0 mg. per cent. The hypocalcemia proved to be refractory to treatment with increasing doses of dihydrotachysterol. She was then given parathormone and when the serum calcium had risen to 10.3 mg. per cent and the phosphorus had fallen to 3.5 mg. per cent all signs of tetany, latent or overt, had vanished. At this time the second adrenaline test was made (Figures 2 and 3).

Patient C. D. (Unit #225260), a fifty-six-year-old white housewife, developed a toxic nodular goiter in 1934. The goiter was removed at this time and six days

after operation she exhibited manifest tetany. Her symptoms were adequately controlled by the continued oral administration of calcium chloride but during the following years there was constant hypocalcemia and hyperphosphatemia. She noted during periods of emotional tension the sudden appearance of tingling of the lips and extremities and not infrequently carpal spasm. When first tested with adrenaline the signs of latent tetany were readily elicited; the serum calcium was 4.9 mg. per cent and the phosphorus was 5.3 mg. per cent. Nine days after treatment with dihydrotachysterol all signs and symptoms of tetany had disappeared; the serum calcium was 9.1 mg. per cent, the serum phosphorus was 5.0 mg. per cent. The adrenaline test was repeated at this time.

Patient F. V. (Unit #242307), a thirty-year-old white female bakery worker gained 40 pounds in weight during 1941 and noted a marked reduction in menstrual bleeding although the cycle was not disturbed. In July, 1941, at the onset of catamenia she developed tingling and cramps in her hands and feet. There was simultaneous swelling of the hands, feet, lips and tongue. Her physician administered adrenaline subcutaneously with prompt subsidence of the localized swellings; but there was an immediate exaggeration of the painful cramps in her extremities and she experienced difficulty in breathing. At each subsequent catamenia the same train of events occurred. She was thought to have "idiopathic" hypoparathyroidism and at the time of the adrenaline test the serum calcium was 5.6 mg. per cent and the serum phosphorus was 5.7 mg. per cent. She was not tested after treatment.

These three patients with tetany, which was apparently of parathyroprivic origin, exhibited similar reactions to the injection of 0.3 mg. of adrenaline into the right brachial artery. The vasomotor responses were similar to those observed in the normal subject but were much more intense. The injected extremity became profoundly blanched and after two or three minutes became cyanotic. The radial pulse vanished. Sweating ceased in the injected arm and hand. Immediately after injection severe, painful, involuntary carpal spasm appeared in the injected hand; the attitude of the hand was tetanic.

After the release of the cuff obstructing the venous return from the extremity, generalized tetany involving the other hand, the facial muscles and the feet became manifest. In one patient, F. V. marked laryngeal stridor was observed. There was extreme blanching of the skin everywhere. The constitutional symptoms produced by the entry of adrenaline into the general circulation were much more severe in these patients than in the normal subjects. On two occasions it became necessary to administer calcium gluconate intravenously

immediately; relief from the general symptoms followed this measure. In one individual, I. W., however, the local phenomena were not altered by the calcium administered. In the other individual, F. V.,

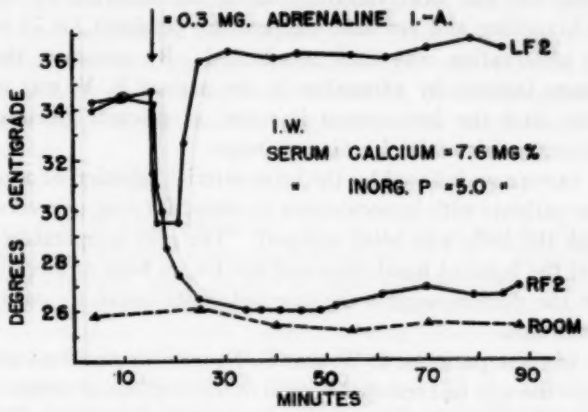

FIG. 2. Patient I. W. Parathyroprivic hypocalcemia. Skin temperatures recorded as in figure 1. Adrenaline (0.3 mg.) injected into the right brachial artery at arrow. Test performed during latent tetany.

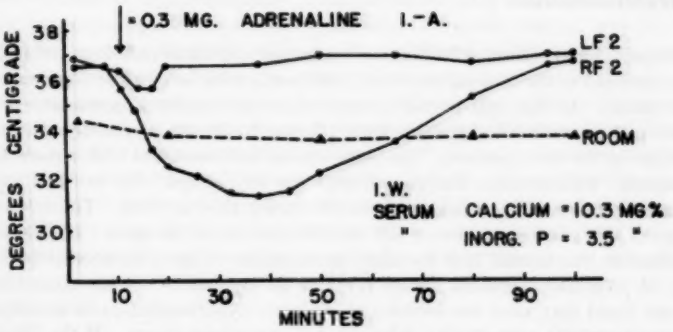

FIG. 3. Patient I. W. Same as figure 2 but after treatment with parathormone. Latent tetany had disappeared with restoration of normal serum calcium.

the intravenous injection of calcium was followed by a rise in skin temperature in the injected hand although the tetanic spasm persisted.

The local tetany persisted for ten to fifteen minutes after the in-

jection of adrenaline; the spasm then relaxed slowly during the next five minutes. It is of considerable interest to note that in the patient I. W. the tetany had disappeared within fifteen minutes after the injection, but the local vasoconstriction, as indicated by the continued blanching and low skin temperature persisted for 75 minutes longer; observation was then terminated. By contrast, the local vasospasm induced by adrenaline in the patient F. V. was released abruptly after the intravenous injection of calcium gluconate but local tetany persisted for 12 minutes longer.

The vasospasm induced by the intra-arterial injection of adrenaline in these patients with hypocalcemia persisted for long periods of time although the body was being warmed. The skin temperature of the digits of the injected hand remained low for an hour or more (Figure 2). In the normal subject the injected digits began to warm in 15 to 30 minutes.

Two of these patients, I. W. and C. D. were re-examined after appropriate therapy had restored normal concentrations of serum calcium and phosphorus. At these times the reactions to adrenaline were indistinguishable from those of normal subjects. There was no tetany and the vasospasm was not persistent (Figure 3).

Hyperventilation:

Patient M. M. (Unit #215237), a twenty-year-old white radiology technician was admitted to the medical service in 1940 shortly after he had had a generalized convulsion. At this time he was discovered to have postural hypotension with a fall in the systolic blood pressure from 110 mm. to 80 mm. of mercury after two minutes in the erect posture. The hypotension was associated with nausea and faintness. This reaction disappeared after one day in bed. He had had acute rheumatic fever with cardiac involvement during 1931 to 1934. The sole evidence of this past process was a soft systolic murmur at the apex. The electrocardiogram was normal save for slight prolongation of the P-R interval to 0.21 sec. A complete diagnostic survey revealed no explanation for the convulsion. It was noted that after two minutes of moderate hyperventilation he developed overt tetany with paresthesiae, faintness and carpopedal spasm. If the circulation to an arm was obstructed by means of a sphygmomanometer cuff inflated to a pressure above the systolic blood pressure, then tetany did not develop in the ischemic extremity (Bordley). When tetany was well developed elsewhere, release of the cuff was not followed by carpal spasm in the ischemic hand. The values of the blood constituents were serum calcium 10.5 mg. per cent; serum phosphorus 4.7 mg. per cent; plasma carbon dioxide combining power 65.6 vols. per cent.

This patient reacted quite normally to the injection of 0.3 mg. of adrenaline into the brachial artery. There was no movement or contraction of the injected extremity, the vasoconstriction was completely released and the skin temperature had returned to its previous high degree within 40 minutes after the injection. The release of adrenaline into the general circulation was not attended by any distress. After this test he began moderate, voluntary hyperventilation and induced well-marked carpopedal and facial tetany within one minute.

Emotional Tension:

Patient S. B. M. (Unit #227962), a nun, thirty years of age, developed hyperthyroidism and underwent a subtotal thyroidectomy in 1923. The postoperative course was uneventful, but in 1929 she developed manifest tetany. At this period the serum calcium is reported to have ranged between 5.8 and 6.7 mg. per cent. She was given various forms of therapy, including parathormone and dihydrotachysterol, with maintenance of normal serum calcium levels and complete freedom from any symptoms. In 1939 during a period of stress the patient developed diarrhea and the tetany returned. There are no determinations of the serum calcium available for this period. In the autumn of 1940 a serious conflict arose in her life situation and shortly thereafter the tetanic symptoms returned. When she was first seen in this clinic in May, 1941, she was tense and had recurrent episodes of apparent tetany. She had been taking dihydrotachysterol and parathormone regularly and the concentration of serum calcium was 10.9 mg. per cent, of serum phosphorus 4.0 mg. per cent, of serum albumin 4.4 gm. per cent and of serum globulin 2.7 gm. per cent. It was noted that the episodes of tetany appeared when the patient was apprehensive although no significant hyperventilation accompanied these attacks. On several occasions it was observed that the Trousseau sign was present but that similar carpal spasm appeared simultaneously in the extremity to which no cuff was applied. When all medication was withheld for three weeks, the serum calcium fell to 8.2 mg. per cent and the serum phosphorus rose to 5.0 mg. per cent and the tetany became more severe. Dihydrotachysterol was administered again, and when the serum calcium had reached 9.5 mg. per cent and the phosphorus 4.7 mg. per cent, the adrenaline test was performed.

This patient showed no tetany, local or generalized, after the injection of 0.2 mg. of adrenaline into the brachial artery. The injected fingers cooled 5 degrees C. and had returned to their former temperature 15 minutes later. During the mild constitutional symptoms which followed the entry of adrenaline into the general circula-

tion, the patient volunteered the information that "this is how I feel during one of my attacks."

Hypercalcemia:

Patient M. C. (Unit #229775), a fifty-six year old white stationary engineer was admitted with symptoms and signs of severe hyperparathyroidism which was proved subsequently to be the result of a large parathyroid adenoma. At the time of the adrenaline test the serum calcium was 17.1 mg. per cent and the phosphorus was 2.9 mg. per cent.

The reaction of this patient to the injection of 0.2 mg. of adrenaline into the brachial artery was indistinguishable from that of the normal subjects.

DISCUSSION

The extraordinary sensitivity to adrenaline which occurred in subjects with hypocalcemia makes it necessary to emphasize the dangers associated with the administration of adrenaline and its related substances. If it be necessary to administer adrenaline to patients with tetany due to hypocalcemia, it is essential first to increase the serum calcium by intravenous exhibition of calcium salts.

The conversion of latent into manifest tetany by emotional tension or exercise has long been noted. This phenomenon occurred in the patients C. D. and I. W. during periods of anxiety. It seems likely that the release of adrenaline during these periods played an important rôle in the development of tetany; hyperventilation which so frequently accompanies anxiety apparently was not an important factor because in these patients tetany could not be induced by voluntary hyperventilation of moderate duration. The sudden release of adrenaline during panic may perhaps explain the sudden death which occasionally occurs in children with tetany. In one instance sudden death occurred when laryngospasm developed during vigorous crying induced by jugular venopuncture (Guild).

The sensitivity to adrenaline which was manifest in both smooth and striated muscle systems in this group of patients apparently was related directly to hypocalcemia. When normal concentrations of serum calcium were attained the sensitivity vanished. Other forms of tetany, not associated with hypocalcemia, showed no sensitivity to adrenaline.

The question arises as to whether the striated muscle spasm produced by adrenaline resulted from the alteration in blood flow. There is no crucial evidence which resolves this question, but it is important to emphasize that, in the patient I. W., tetany disappeared many minutes before the vascular spasm was released; and, conversely, in the patient F. V. local tetany was present 12 minutes after the release of vascular spasm. This suggests strongly that the two phenomena were coincidental rather than casually related. This opinion was expressed by v. Frankl-Hochwart (1889).

These observations re-emphasize the rôle played by environmental chemical changes in the maintenance of normal neuromuscular function. A large body of data has been accumulated regarding the effect on excitable tissues of alterations in the ionic pattern of the fluid environment. With the exception of a few special instances, tissue excitability is in general enhanced by diminished concentrations of available calcium ions. The effect of changes in the concentration of calcium is, however, related to concomitant changes in the concentration of potassium ions and the resultant alteration of the potassium-calcium ratio; for these two ions produce antagonistic effects. These general phenomena have been studied recently in specific neural (Bronk, 1939), muscular (Harvey, 1940), neuromuscular (Harvey and Wigton, 1940), and vasomotor systems (Jang, 1940). It has been shown that the usual behaviour of excitable tissues under conditions of varying potassium-calcium ratios is reflected in these special systems. The recent studies of these systems have demonstrated that the responses to stimulation by "chemical" mediators such as potassium, acetylcholine and adrenaline are greatly enhanced by a reduction in the available calcium in the environment. Since adrenaline stimulates structures innervated by adrenergic components of the autonomic nervous system, it seems likely that the extreme vasomotor responses to adrenaline observed in these patients with hypocalcemia resulted from such a sensitizing process and correspond to Jang's (1940) observations on the vessels of the rabbit ear.

In contrast to the exaggerated vasomotor effect, the mechanism of the response of striated muscle (tetany) to adrenaline is not clear. Fenn (1940, 1941) has outlined the many and conflicting data regarding the rôle played by potassium in muscle contraction and neuro-

muscular transmission. There are, in addition, many observations upon the effect of adrenaline on the movement of potassium ions between various fluid compartments in the organism. Both Castleden (1938) and Keys (1938) have shown that adrenaline regularly produces a fall in the concentration of serum potassium in man. The evidence which relates changes in the potassium-calcium ratio to tissue excitability has been indicated above. These data may well contain the answer to the observations which we have recorded here, but any speculation would ignore the fact that the ionic shifts which may occur in the muscle fibers and at the neuromuscular junction are unknown. It may be suggested merely that the tetany produced by adrenaline resulted from an abrupt distortion of the potassium-calcium ratio.

The effect of adrenaline in this group of patients brings out an important difference between hypocalcemic and hyperventilation tetany. Although Duzár and Hensch (1926) showed that normal children, after 5 or 6 minutes of voluntary hyperventilation, could be rendered actively tetanic by the intravenous injection of adrenaline without further hyperventilation, it will be noted that preliminary hyperventilation was necessary for the development of "adrenalintetanie." In the subjects studied here the differentiation of hyperventilation from hypocalcemic tetany by the absence of sensitivity to adrenaline was made without preliminary hyperpnea. Thus although the changes in electrolyte pattern produced by adrenaline will evoke tetany in either type under suitable circumstances, when no preliminary hyperventilation is induced the response to the administration of adrenaline can be used to differentiate between latent hyperventilation tetany and hypocalcemic tetany.

The observations we have recorded make it appear that the site of the tetanic reaction is primarily peripheral in both hypocalcemic and hyperventilation tetany. Hyperventilation tetany does not appear in the hand from which the circulation has been excluded prior to the hyperpnea. This makes unlikely the possibility that hyperventilation tetany depends upon any changes occurring centrally in the nervous system. It is true, however, that any changes in the electrolyte pattern produced by ischemia in the environment of the peripheral structures have not been assayed; and the possibility exists

that such changes might mask or prevent the development of local tetany. The Trousseau phenomenon develops in the hand of the patient with latent hypocalcemic tetany even though the motor nerves have been blocked with procaine below the cuff (unpublished observations). This suggests that the site of this disturbance is likewise peripheral.

SUMMARY

1. The injection of adrenaline into the brachial artery of normal subjects produced vasoconstriction in the injected extremity.

2. The intra-arterial injection of adrenaline in three patients with hypocalcemia evoked immediate local tetany and prolonged local vasoconstriction. These two manifestations were apparently independent of each other.

3. Normal vasomotor reactions to adrenaline were noted in one patient with hyperventilation tetany, in another patient with tetany evoked by emotional tension, and in a third patient with extreme hypercalcemia.

4. The development of adrenaline sensitivity in the hypocalcemic patient is similar to the lowered threshold to chemical mediators observed in a variety of excitable systems placed in an hypocalcemic environment. The potassium-calcium ratio and the effect of adrenaline on the migration of potassium ions are probably related to the development of this sensitivity.

5. The danger attending the administration of adrenaline to hypocalcemic patients is stressed.

We are greatly indebted to Dr. Charles A. Ragan, Jr., and Miss Margot Robinson for the determinations of skin temperatures.

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THE RELATIONSHIP BETWEEN SERUM CHOLESTEROL AND TOTAL BODY CHOLESTEROL IN EXPERIMENTAL HYPER- AND HYPOTHYROIDISM[1]

WALTER FLEISCHMANN AND HARRIS B. SHUMACKER, JR.

With the technical assistance of EDWARD SMETANA

From the Department of Pediatrics and the Surgical Hunterian Laboratory, The Johns Hopkins University School of Medicine and the Harriet Lane Home, The Johns Hopkins Hospital

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The existence of a relationship between thyroid function and blood cholesterol level is generally accepted. The mechanisms involved in this relationship are little understood, however. Last year we reported sterol balance experiments on patients whose diet was practically free from cholesterol (1). Studies on two cretins and one dwarf without evidence of hypothyroidism showed that the characteristic changes in the concentration of serum cholesterol, which occur on the institution or withdrawal of thyroid treatment are not affected by the lack of cholesterol in the diet. The studies showed further that the drop in serum cholesterol due to thyroid administration is not associated with an increased excretion of cholesterol through the intestines. The rise of serum cholesterol following the withdrawal of therapy is not due to a retention of cholesterol.

If absorption from or excretion through the intestines can be excluded as factors involved in the thyroid-serum cholesterol relationship, two possible explanations remain. Changes in the cholesterol level of the serum might indicate either a change in the total cholesterol content of the body or a shift of cholesterol between the blood plasma and the tissues. In the first case, an increased cholesterol destruction at the onset of thyroid treatment and an increased synthesis on withdrawal of thyroid would have to be postulated, or in other words, an influence of the thyroid on cholesterol metabolism. In the second case,

[1] Reported (by title) at a meeting of the Association for the Study of Internal Secretions, June 8th, 1942, Atlantic City.

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the thyroid hormone might act on the distribution of cholesterol in the body rather than on the metabolism of cholesterol. To determine which possibility is the more probable, we have studied the serum cholesterol and total body cholesterol in rats treated with thyroxin, in thyroidectomized rats and rabbits, and in normal controls of both species.

The results of similar studies made by others on total body cholesterol have been contradictory. It has been stated by Vignes and Glomaud (2) that administration of thyroxin brings about no change in the total body cholesterol content of rats. On the other hand, the experiments of Hurxthal and Perkin (3) seemed to show that the body content of cholesterol in mice was decreased by the feeding of desiccated thyroid and that this decrease was not due to increased excretion.

MATERIALS AND METHODS

Rabbits were fed a diet of alfalfa and oats.

Rats were fed a diet of the following formula: Glucose, 260 cc.; dried brewers' yeast 150 cc.;[2] casein, washed, 150 cc.; olive oil 50 cc.; NaCl 5 gm.; KCl 5 gm.; calcium lactate 12 gm.; Na_2HPO_4 20 gm.; $MgCl_2$ 5 gm.; carotene (Smaco) 6 drops.

Total body cholesterol was determined in the exsanguinated animals by the method of Schoenheimer and Breusch (4). Serum cholesterol was determined by the colorimetric method using an Evelyn photoelectric colorimeter (5). The animals were kept on the diet for one week before the beginning of the experiment.

RESULTS

Experiments on Thyroidectomized Rabbits

In table 1 are shown the results of thyroidectomy in four rabbits of about 5 weeks of age. In all of them hypercholesterolemia developed rapidly after operation as reported previously (6). Three of these animals (Nos. 3, 4, 5) were sacrificed at the same time as two normal rabbits of about the same age (Nos. 1, 2). The intestines were removed and the total body cholesterol determined. Although the serum cholesterol level had been more than doubled in the operated animals, the concentration of cholesterol in the body was practically unchanged. The fourth animal of the operated group (No. 6) whose serum cholesterol one week after operation was 190 mg. per cent, was

[2] The dried yeast and casein were measured in cubic centimeters.

treated for a week with thyroxin. The total amount administered was 0.4 mg. The rabbit showed loss of weight and other signs of hyperthyroidism. It was sacrificed and was found to have the highest percentage of total body cholesterol (.242) and the lowest serum cholesterol, 58 mg. per cent, of all the rabbits analyzed.

TABLE 1

Effect of thyroidectomy on total body and serum cholesterol of rabbits

NO.	WEIGHT	TOTAL BODY CHOLESTEROL	CHOLESTEROL, PER CENT OF BODY WT.	SERUM CHOLESTEROL	
	gm.	*mg.*		*mg. per cent*	
1	463	780	.168	88	Normal
2	360	525	.146	98	Normal
3	450	735	.163	203	Thyroidectomized
4	475	780	.164	230	Thyroidectomized
5	653	915	.140	236	Thyroidectomized
6	248	600	.242	58	Thyroidectomized, then treated with thyroxin

TABLE 2

Effect of thyroidectomy on total body and serum cholesterol of rats

RAT NO.	AGE WHEN KILLED	WEIGHT	TOTAL BODY CHOLESTEROL	CHOLESTEROL, PER CENT OF BODY WEIGHT	SERUM CHOLESTEROL	
	days	*gm.*	*mg.*		*mg. per cent*	
11	43	119	210	.18	86	Normal
12	43	123	200	.16	75	Normal
13	43	79	170	.22	76	Thyroidectomized
14	43	50	110	.22	136	Thyroidectomized
15	43	79	170	.22	131	Thyroidectomized
16		161	393	.24	81	Normal
17		166	380	.22	203	Thyroidectomized

Experiments on Thyroidectomized Rats

In table 2 are shown the effects of thyroidectomy on three rats (Nos. 13, 14 and 15) operated on at the age of 27 days and sacrificed 16 days later, as compared with the unoperated controls (Nos. 11 and 12) killed at the same age. In addition, an older rat (No. 17) in which

hypercholesterolemia developed four weeks after thyroidectomy is compared with a normal rat of about the same weight (No. 16).

In this series of experiments there were two animals (Nos. 14 and 15) in which the serum cholesterol was elevated (over 100 mg. per cent) and one animal (No. 13) in which normal cholesterol was found. The fact that thyroidectomy in the rat is not always followed by hypercholesterolemia has been noted by others (7).

It is of interest that both the body weight and the total body cholesterol were reduced in the young thyroidectomized rats (Nos. 13, 14, 15) as compared with the unoperated controls (Nos. 11, 12) of the same age. The cholesterol content in percentage of body weight was, however, higher in the operated animals. This finding will be discussed later. In the older rat (No. 17) the result was comparable to that in the rabbits. Although the serum cholesterol was 203 mg. per cent in the operated rat (No. 17), as against 81 mg. per cent in the control (No. 16), the concentration of body cholesterol was .22 in No. 17 and .24 in No. 16.

Experiments on Rats Treated with Thyroxin

In table 3 are shown the results of experiments on rats treated with thyroxin. In the first two litters of rats of 28 days (Nos. 21 to 25) and of 33 days of age (Nos. 31 to 37), respectively, one litter mate was killed at the outset of the experiment and the rest of the litter was divided into two lots. Half the rats were treated with thyroxin for 14 days and then killed; the others were not treated and were killed at the same time. Thyroxin (0.7 to 1.0 mg.) was injected every other day, so that the treated animals received a total of from 5.9 to 6.8 mg. thyroxin.

In a third litter three animals aged 19 days (Nos. 44, 45, 46) were treated with a total of 4 mg. thyroxin during two weeks; the other three animals in the litter (Nos. 41, 42, 43) were used as controls. In a fourth litter (Nos. 51 to 56), two animals (Nos. 51, 52) were killed at the age of 26 days; two animals (Nos. 55, 56) were treated with doses of 0.12 mg. thyroxin every other day so that they received a total of 0.9 mg. during two weeks. The other two animals (Nos. 53, 54) were killed at the end of that time as controls. In the animals of litter four receiving these comparatively small doses of thyroxin, no effect on body

weight, serum cholesterol or activity was observed when compared to the untreated litter mates.

In litters one and two of this series it can be noted that the increase in weight was considerably greater during the same period in the un-

TABLE 3

Effect of thyroxin on total body and serum cholesterol of rats

LITTER	RAT NO.	TREATMENT	BEFORE B		WHEN KILLED		WT. INCREASE DURING B	TOTAL BODY CHOLESTEROL	CHOLESTEROL INCREASE DURING B	CHOLESTEROL, PER CENT OF BODY WT.	SERUM CHOLESTEROL
			Age	Wt.	Age	Wt.					
		mg.	*days*	*gm.*	*days*	*gm.*	*gm.*	*mg.*	*mg.*		*mg. per cent*
1	21		28	46	28	46		90		.19	73
	22	0	28	54	42	83	29	150	60	.18	90
	23	0	28	49	42	83	34	160	70	.19	80
	24	Thyroxin 6.8	28	50	42	59	9	140	50	.24	69
	25	Thyroxin 6.7	28	50	42	54	4	150	60	.28	64
2	31		33	60	33	60		130		.22	95
	32	0	33	52	47	93	41	170	40	.18	85
	33	0	33	58	47	102	44	190	60	.19	94
	34	0	33	60	47	104	44	170	40	.16	74
	35	Thyroxin 6.5	33	62	47	84	22	180	50	.21	40
	36	Thyroxin 5.9	33	62	47	66	4	160	30	.24	40
	37	Thyroxin 5.9	33	62	47	68	6	150	20	.22	52
3	41	0	19	44	33	72	28	177		.24	80
	42	0	19	43	33	71	28	165		.23	95
	43	0	19	45	33	76	31	162		.21	89
	44	Thyroxin 4.0	19	45	33	59	14	147		.25	42
	45	Thyroxin 4.0	19	45	33	56	11	152		.27	57
	46	Thyroxin 4.0	19	45	33	58	13	142		.24	55
4	51		26	38	26	38		100		.26	40
	52		26	41	26	41		95		.23	44
	53	0	26	39	40	89	50	215	117	.24	62
	54	0	26	39	40	94	55	205	107	.22	62
	55	Thyroxin 0.9	26	39	40	85	46	220	122	.26	62
	56	Thyroxin 0.9	26	39	40	85	46	200	102	.24	62

treated animals than in those given thyroxin. Between the 28th and 42nd day the weight of the untreated rats (Nos. 22 and 23) increased 29 and 34 grams, respectively, while the treated litter mates (Nos. 24 and 25) gained only 9 and 4 grams, respectively. If we consider that

the total body cholesterol was approximately 90 mg. on the 28th day as found in rat No. 21, the amount of body cholesterol synthesized between the 28th and 42nd day averaged 65 mg. in the normal, 55 mg. in the treated group.

The intestines were cleaned of their intestinal contents in all rats in which determinations of total body cholesterol were done. In litter one the stools were collected separately for each animal over the 14-day period and sterols determined for the combined intestinal contents and stools by the same method as in the tissues. As the food was practically free from sterols the sterol found in the feces was considered to be excreted cholesterol or its derivative, coprosterol. The amounts of cholesterol excreted were 13 mg. and 12 mg., respectively, in the untreated (Nos. 22 and 23), and 10 mg. and 8 mg., respectively, in the rats treated with thyroxine (Nos. 24, 25). The total amount of cholesterol (body cholesterol plus stool cholesterol) synthesized was 77 mg. in the normal, 64 mg. in the treated group.

In the second litter the weight increase in the untreated groups averaged 43 grams; in the treated group 11 grams. Using 130 mg. found in rat No. 31 on the 33rd day as the base level, the average amount of cholesterol synthesized was 47 mg. in the normal, 33 mg. in the treated group. Since the total cholesterol continued to increase during treatment with thyroxin, even though the gain in body weight was greatly diminished, it is not surprising that in all of the litters examined the cholesterol content in per cent of body weight was higher in the thyroxinized animals than in the untreated controls. The serum cholesterol level was lower in the animals receiving large doses of thyroxin as compared with the controls. In only the last litter (Nos. 51 to 56) was there no difference in serum cholesterol between the treated and the untreated group, but in this experiment the dose of thyroxin was considerably smaller.

We have combined the data for the first three litters of rats because the amount of thyroxin given was of the same order of magnitude in the three experiments. It appears that the mean cholesterol concentration for the treated rats was .244 $\pm$ .006 per cent of the body weight, whereas it was only .198 $\pm$ .006 per cent in the control animals. The difference is +.046 $\pm$ .009 per cent. For serum cholesterol, on the other hand, the mean values were 52.4 $\pm$ 1.8 mg. per cent for the

treated rats and 85.9 ± 2.6 mg. per cent for the untreated controls. The difference is −33.5 ± 3.2 mg. per cent and represents a decrease rather than an increase as was the case with the concentration of total body cholesterol. The differences are five and ten times their probable errors, respectively, and therefore are to be considered statistically significant.

DISCUSSION

The experiments in the rabbit in which the removal of the thyroid resulted in a marked hypercholesterolemia without any change in the cholesterol content of the body demonstrate clearly that the blood cholesterol level does not reflect the cholesterol content of the body. The experiments in rats, both in thyroidectomized and thyroxin-treated animals, show no parallelism between cholesterol level of the serum and total cholesterol content of the body.

In the rat both hypothyroidism and hyperthyroidism lead to a higher cholesterol concentration in the body (in percentage of body weight) as compared to that of normal controls. This fact may be explained by the finding that both thyroidectomy and thyroid feeding result in a marked reduction of body fat in the rat. Mackay and Sherrill (7) have reported that body fat (total lipids) is as low as 6.4 per cent of body weight in thyroidectomized rats and is 14.2 per cent in thyroid fed rats as compared with 31.1 per cent in unoperated controls. Fatty tissues contain no cholesterol or only very small amounts. Therefore a marked reduction in the amount of these tissues is equivalent to a proportionate increase of tissue containing cholesterol, and to an increase of the cholesterol concentration in the body.

It was shown in our previous paper (1) that the changes in the concentration of serum cholesterol in hypothyroidism and hyperthyroidism do not depend upon alterations in the absorption or excretion of cholesterol. The experiments reported here indicate that the changes in the concentration of cholesterol in the serum are not parallel to changes in the concentration of cholesterol in the body. Apparently the changes in the serum cholesterol depend upon the degree to which the substance is shifted into the blood plasma from its storage depots, or removed from the blood stream. Cholesterol is believed to exert a carrier function for fatty acids in the blood (8). It has been shown by

a number of workers (9, 10, 11, 12) that there is a fairly constant ratio between free and total cholesterol and between total cholesterol and total fat in the plasma under numerous conditions including changes in thyroid activity. It is probable therefore that the alterations of serum cholesterol merely reflect the rate at which fatty acids are being transported for oxidation or storage.

SUMMARY

Total body cholesterol and serum cholesterol were determined in thyroidectomized rabbits and rats, in rats treated with thyroxin and in normal controls. It was found that changes in the concentration of cholesterol in the serum do not parallel changes in the concentration of cholesterol in the body. The experimental data indicate that the thyroid hormone influences the shift of cholesterol to and from the blood plasma rather than that it has a specific effect on cholesterol metabolism.

We wish to thank Dr. Christopher Tietze of the Johns Hopkins University School of Hygiene for his help with the statistical evaluation of our data.

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BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal.)

Gynecological and Obstetrical Pathology. By EMIL NOVAK. 496 pp. Illus. $7.50. W. B. Saunders Company, Philadelphia and London, 1940.

Once more the indefatigable worker, Dr. Novak, has made a contribution to the medical literature. This is probably the most valuable of all his literary productions for it embodies his life work, pathology. As he says, this book is based chiefly on his work in the Laboratory of Gynecological Pathology in the Johns Hopkins Hospital during the past twenty-five or more years. During a large part of that time he has been in charge of the Laboratory and the teaching of Gynecological Pathology to the students. During those years, in addition to the many thousands of specimens that are routinely sent from the gynecological operating room of the hospital, specimens of many rare lesions have been sent from other hospitals to the Laboratory for diagnosis and pathological study. It is fitting, therefore, that the experience of the Laboratory should be presented in a book, and no one is as well qualified to present it as Dr. Novak.

The book is comprehensive and complete and covers practically every known disease of the female reproductive system. The subject is treated systematically and is presented from a clinical viewpoint so that the student may be able to correlate the pathology with the clinical features. There is also liberal consideration of the physiological features of the various organs, for without that the study of many gynecological lesions would be incomplete.

The reviewer has been using the book since it appeared and can add no greater compliment than to say that it is the outstanding book on this subject.

L. R. W.

Clinical Hematology. By MAXWELL M. WINTROBE. 792 pp. Illus. $10.00. Lea and Febiger, Philadelphia, 1942.

This book is an excellent comprehensive survey of the diseases of the blood. The subject is well covered, both from the clinical and hematological standpoints. The usual technical procedures for making hematological examinations, their significance and their limitations, are given in sufficient detail to be useful to the beginner. Those subjects to which the author himself has made important contributions are especially valuable, such for example as the determination of the volume and concentration of hemoglobin in the red blood cells and the sedimentation rate.

The book, however, is much more than a text for students. In addition to the topics usually covered in such a book, a great variety of rare and unusual conditions receive at least a brief mention. Very little of importance has been omitted. The book is not padded, however, and obsolete and unimportant material has been excluded.

It is well illustrated, and there are seven colored plates showing the appearance of all the ordinary types of normal and pathological blood cells in stained films.

One of the most valuable features of the book is the extensive and carefully chosen bibliography which follows every chapter. This serves adequately to document practically every condition discussed, and will be of great assistance to anyone who wishes to pursue the subject further.

The book is admirable for the clarity with which it is written and for its nearly flawless English. It is a valuable contribution and is highly recommended.

P. W. C.

Eye Manifestations of Internal Diseases. By I. S. TASSMAN. 542 pp. Illus. $9.50. The C. V. Mosby Company, St. Louis, 1942.

This volume contains 498 pages of printed material amongst which there are scattered 182 illustrations in black and white. In addition there are 19 colored plates most of which portray optic fundus conditions. The subject matter has been divided into 20 chapters. Chapters I–V inclusive, provide descriptions of normal ocular and orbital structures for the most part, and these chapters also contain information regarding methods of examination of the eyes. Chapters VI–XX inclusive, contain information all of which is entirely pertinent to the title of the volume.

The book contains much useful information. The arrangement of the clinical material dealing with the ocular signs of internal diseases has been carefully thought out. The author has a pronounced ability to condense information and the entire volume reflects this attainment.

The black and white illustrations have for the most part been carefully selected and in most instances the reproductions are good. The colored illustrations are almost all excellent. There are an unusual number considering the small size of the volume. The reviewer cannot see any possible value, however, in inserting illustrations of such objects as ophthalmoscopes, perimeter and loupe. Surely such illustrations serve no useful purpose.

This reviewer has two criticisms, and regarding the first of these there may not be general agreement. In the first place, much of the material presented is not relevant to the title of the book. Secondly, some of the descriptions do not contain all the available information; for example the sections on intracranial tumors, aneurysms, migraine and hysteria, to mention only a few.

F. B. W.

The Toxemias of Pregnancy. By WILLIAM J. DIECKMANN. 521 pp. Illus. $7.50. *C. V. Mosby Company, St. Louis, 1941.*

Dr. Dieckmann's views on the engrossing topic of the toxemias of pregnancy have long attracted favorable attention. In the present volume he has not only summarized his own opinions, which are based on much original work, but he has assembled and correlated a huge amount of data from the world's literature. The result is a book which presents a clear and well organized account of our

current knowledge of the subject, seasoned at intervals by the author's own ideas and policies. It is the first such survey to appear since that of Stander was published some thirteen years ago.

The work was written with two objectives in mind: (1) to acquaint the obstetrician with some of the recent contributions on physiology pertaining to the toxemias, and (2) to acquaint the investigator untrained in obstetrics with some of the clinical aspects of the toxemias. In the pursuit of the first of these ends detailed consideration is given to the pathology of eclampsia and most exhaustive surveys are presented on physico-chemical aspects, blood pressure, renal physiology, the ocular system and on hepatic physiology and pathology. The elusive enigma of the cause of the toxemias is the subject of several chapters. The author is skeptical alike of endocrine explanations and of placental infarct theories and wisely leaves the question unanswered.

The portion of the book dealing with clinical aspects of the toxemias is replete with illustrative case histories which are examples of careful clinical observation and meticulous laboratory study. This section reviews many older forms of therapy, now abandoned, and cites figures to show why they were given up. Today, when a tendency has developed to revive some of these, notably veratrum viride, these old experiences are worth recalling. Particular emphasis is laid on the value of hypertonic glucose solutions in the treatment of eclampsia for stimulating diuresis and reducing hemoconcentration. An interesting historical account of eclampsia concludes this section.

In sum, this book is a commendable and welcome achievement and deserves a place on the book shelf of every physician who practices obstetrics.

N. J. E.

Methods of Treatment. By LOGAN CLENDENING and EDWARD H. HASHINGER. 7th edition. 997 pp. Illus. $10.00. *The C. V. Mosby Company, St. Louis, 1941.*

This new edition of Clendening's work has been extensively revised, portions of it entirely rewritten, and considerable new material has been added. The general plan and scope of the book, however, have not been changed.

Among the more important additions and revisions are the sections on anesthetics, radiotherapy, diathermy, artificial pneumothorax, vitamins and deficiency diseases, endocrine disturbances and their specific treatment, and the use of sulfanilamide and related drugs. The sections on allergy and cardiac failure have been revised and improved. A good, new section on peripheral vascular diseases has been added. Among the subjects dropped are the use of tuberculin, bacteriophage, and malarial inoculations in the treatment of syphilis. More attention has been given to physical methods of inducing hyperpyrexia.

The author has retained his conservatism in omitting new drugs and procedures, the value of which has not been demonstrated. In general, however, there are few omissions of significance and the book has been brought well up to date. The high standard of previous editions has been retained.

P. W. C.

Oral Pathology. By KURT H. THOMA. 1306 pp. Illus. $15.00. *The C. V. Mosby Company, St. Louis, 1941.*

Oral Pathology, By Dr. Kurt H. Thoma, is a comprehensive volume which includes studies on experimental pathology, developmental anomalies of the teeth and head, functional changes and traumatic injuries of the teeth, odontitis and odontogenic infection, and diseases of the hard and soft structures of the mouth.

The author, who is an accomplished oral pathologist, has presented an excellent reference work for graduate students in a field which requires years of clinical experience for its proper understanding and evaluation. The mouth is considered as a part of the body as a whole. Pathology, rather than therapy, is the primary consideration.

The book is unique in that it is the first of its kind to conform to the nomenclature adopted by the Academy of Periodontology in Cleveland, 1940.

Numerous good dental books have appeared in recent years but it is only more so a compliment to say that Dr. Thoma's new one belongs on the "must list". One feels that a book of this type will not be surpassed for years to come. If one sees and peruses it, recommendation is scarcely necessary.

B. L. B.
L. W. G.

Diseases of the Respiratory Tract. By JACOB SEGAL. 172 pp. $2.00. Oxford University Press, London, New York, Toronto, 1941. (Oxford Medical Outline Series.)

It is hard to see just what purpose this outline of all types of affections of the respiratory tract will serve. Its author evidently offers this more as a review and a supplement to more extensive reading; and parts of the book, particularly the very good outline of pulmonary tuberculosis, as well as of carcinoma of the lung, silicosis and lobar pneumonia can be recommended from that standpoint. The chief weakness of the rest of the book is that in order to tabulate the features of a disease or condition in one, two, three order the controversial aspects of the subject are usually lost sight of and the reader is left with an oversimplified picture that is often misleading to say the least. For example, we find bronchiectasis and pulmonary fibrosis listed as two of the three sequelae of influenza; the first item listed under "Laboratory Findings" in Asthma is "leucocytosis due to eosinophilia"; acute follicular tonsillitis and "epidemic follicular tonsillitis" are described separately though due to the same organisms. One also gets the impression that the etiology of bronchiectasis is practically settled and can nearly always be traced to bronchopneumonia in childhood complicated by areas of atelectasis. Lung abscess is discussed separately from gangrene of the lung; and surgery is not mentioned for treatment of "pyogenic diseases of the lung" whereas in the cases of gangrene that have not reached the chronic stage we are told that if the cavity is well localized the operation is "free from risk" and offers a "thorough cure of lung gangrene." This is a most optimistic picture, and an example of why the whole book leaves one somewhat confused.

A. M. F.

Essentials of Pharmacology and Materia Medica for Nurses. By ALBERT J. GILBERT and SELMA MOODY. 251 pp. Illus. $2.25. *C. V. Mosby Company, St. Louis, 1941.*

This is a concise, elementary textbook prepared for a thirty-hour course in a school of nursing, with the object of eliminating "the multitude of relatively worthless proprietary remedies now on the market" and to include those approved by recognized authorities.

The authors have taught the subject in schools of nursing and are familiar with the problems inherent in the subject. Each important drug is briefly discussed according to its action, use, dosage, administration and untoward effects, in a very clear way and without repetition. It seems illogical, however, to include in the chapter on drugs and solutions an arithmetical review and relegate to the appendix the tables of the metric and apothecaries' system which practically every student needs to review.

There are several beautiful colored plates and a number of useful diagrams showing the action of drugs.

This little volume should be very helpful to an instructor and students in selecting material to be learned, or for a quick review. References are given to more extensive works which can be used with it if desired.

L. E. K.

Die Brücke. Klinische Bilder in sechs Sprachen. By FRITZ LEJEUNE. 690 pp. RM. 18. Georg Thieme, Leipzig, 1941.

This book is meant to help the practitioner in talking with patients from abroad. A number of imaginary conversations in German, English, French, Italian, Spanish and Portuguese deal with such diseases as are most likely to come to the physician's attention. From the words of welcome to the examination, diagnosis and treatment, and down to the matter of fees, every little detail is carefully pictured. A subject index makes it easy to find the particular ailment in which the doctor is interested. The text given does not sound bookish but rather idiomatic. the German author has been supported in his task by the collaboration of experts from those countries into whose language the German original has been translated.

The aim of the book is praiseworthy. Even those who are able to read foreign languages fluently often find it difficult to express themselves in such a way that the full impact of their words can readily be grasped. However, from the point of view of the doctor no less than from that of the patient, it is of the utmost importance that the advice given be easily and adequately understood. It is a sad irony that a book meant to bridge the gulf between nations should have come out in the second year of this war. For some time to come it will be useful only for that purpose for which it is meant "last but not least", as the author says (p. 141): for patients and doctors in military hospitals.

L. E.

BOOKS RECEIVED FOR REVIEW

Doctor Bard of Hyde Park. By JOHN BRETT LANGSTAFF. 365 pp. Illus. $3.75. *E. P. Dutton & Co., New York, 1942.*

Biology of the Negro. By JULIAN HERMAN LEWIS. 433 pp. $5.00. *University of Chicago Press, Chicago, 1942.*

Management of the Sick Infant and Child. By LANGLEY PORTER and WILLIAM E. CARTER. 6th ed. 977 pp. Illus. $11.50. *The C. V. Mosby Company, St. Louis, 1942.*

Johns Hopkins Hospital. Dietary Department. Manual of Applied Nutrition. 2nd printing. 79 pp. $1.00. *The Johns Hopkins Hospital Dietary Department, Baltimore, 1941.*

Synopsis of Ano-Rectal Diseases. By LOUIS J. HIRSCHMAN. 2nd ed. 315 pp. Illus. $4.50. *The C. V. Mosby Company, St. Louis, 1942.*

Spontaneous and Experimental Leukaemia in Animals. By JULIUS ENGELBRETH-HOLM. Translated from Danish into English by C. L. Heel. 245 pp. Illus. 15 shillings. *Oliver and Boyd, Edinburgh and London, 1942.*

Carcinoma and Other Malignant Lesions of the Stomach. By WALTMAN WALTERS, HOWARD K. GRAY, and JAMES T. PRIESTLEY. 576 pp. Illus. $8.50. *W. B. Saunders Company, Philadelphia and London, 1942.*

Chemistry and Physiology of the Vitamins. By H. R. ROSENBERG. 674 pp. Diagrams. $12.00. *Interscience Publishers, New York, 1942.*

Pain. By SIR THOMAS LEWIS. 192 pp. Illus. $3.00. *The Macmillan Company, New York, 1942.*

History of the School of Nursing of the Presbyterian Hospital, New York, 1892–1942. By ELEANOR LEE. 286 pp. Illus. $3.50. *G. P. Putnam's Sons, New York, 1942.*

Collected Papers of the Mayo Clinic and the Mayo Foundation. Edited by RICHARD M. HEWITT [and others]. Vol. 33, 1941. 1099 pp. Illus. $11.50. *W. B. Saunders Company, Philadelphia and London, 1942.*

Health Education of the Public. By W. W. BAUER and THOMAS G. HULL. 2nd ed., 315 pp. Illus. $2.75. *W. B. Saunders Company, Philadelphia and London 1942.*

Personality and Mental Illness. By JOHN BOWLBY. 280 pp. $2.75. *Emerson Books, New York, 1942.*

Psychologic Care During Infancy and Childhood. By RUTH MORRIS BAKWIN and HARRY BAKWIN. 317 pp. Illus. $3.50. *D. Appleton-Century Company, New York, 1942.*

1942 Directory of Planned Parenthood Services. 8 pp. *Planned Parenthood Federation of America, Inc., New York, 1942.*

Drugs and Mental Disease. A Review of 841 cases. By MERRILL MOORE, ALICE F. RAYMOND, and M. G. GRAY. 41 pp. Reprinted in the United States of America from *Confinia Neurologica*, Volume 4, pages 238–271, 1941.

Blood Grouping Technic. By FRITZ SCHIFF and WILLIAM C. BOYD. 248 pp. Illus. $5.00. *Interscience Publishers, New York, 1942.*

The Dynamic State of Body Constituents. By RUDOLF SCHOENHEIMER. 78 pp. $1.75. *Harvard University Press, Cambridge, Mass., 1942.*

Management of Fractures, Dislocations, and Sprains. By JOHN ALBERT KEY and H. EARLE CONWELL. 3rd ed. 1303 pp. Illus. $12.50. *The C. V. Mosby Company, St. Louis, 1942.*

Manual of Standard Practice of Plastic and Maxillofacial Surgery. Prepared and edited by the Subcommittee on Plastic and Maxillofacial Surgery of the Committee on Surgery of the Division of Medical Sciences of the National Research Council, and Representatives of the Medical Department, U. S. Army. 432 pp. Illus. $5.00. *W. B. Saunders Company, Philadelphia & London, 1942.*

Principles and Practice of Cardiology. By CRIGHTON BRAMWELL and JOHN T. KING. 509 pp. Illus. $12.00. *Oxford University Press, London: Humphrey Milford, 1942.*

Biological Symposia. Volume VII. *Visual Mechanisms.* Edited by HEINRICH KLÜVER. 322 pp. Illus. $3.25. *The Jaques Cattell Press, Lancaster, Pa., 1942.*

Sulfonamide Compounds in the Treatment of Infections. By MAURICE A. SCHNITKER. 195 pp. $2.00. *Oxford University Press, New York [etc.], 1942.* (Reprinted from Oxford Loose-Leaf Medicine.)

Essentials of Pathology. By LAWRENCE W. SMITH and EDWIN S. GAULT. 2nd ed. 960 pp. Illus. $10.00. *D. Appleton-Century Company, New York and London, 1942.*

Treatment in General Practice. By HARRY BECKMAN. 4th ed. 1015 pp. $10.00 *W. B. Saunders Company, Philadelphia and London, 1942.*

War Gases. By MORRIS B. JACOBS. 180 pp. Diagrams. $3.00. *Interscience Publishers, New York, 1942.*

Morris' Human Anatomy. 10th ed. Edited by J. PARSONS SCHAEFFER. 1635 pp. Illus. $12.00. *The Blakiston Company, Philadelphia, 1942.*

Interaction of Drugs and Cell Catalysts. By FREDERICK BERNHEIM. 85 pp. $2.25. *Burgess Publishing Co., Minneapolis, 1942.*

Diseases and Injuries of the Larynx. By CHEVALIER JACKSON and CHEVALIER L. JACKSON. 2nd ed. 633 pp. Illus. $8.00. *The Macmillan Company, New York, 1942.*

Psychotherapy in Medical Practice. By MAURICE LEVINE. 320 pp. $3.50. *The Macmillan Company, New York, 1942.*

Surgery of Pancreatic Tumors. By ALEXANDER BRUNSCHWIG. 416 pp. Illus. $7.50. *The C. V. Mosby Company, St. Louis, 1942.*

Care of the Aged (Geriatrics). By MALFORD W. THEWLIS. 4th ed. 589 pp. Illus. $7.00. *The C. V. Mosby Company, St. Louis, 1942.*

SUCCESSFUL CONSTRUCTION OF AN EXTRATHORACIC ESOPHAGUS*

JOHN STAIGE DAVIS, M.D., AND EDWARD S. STAFFORD, M.D.

From the Department of Surgery, The Johns Hopkins University and the Johns Hopkins Hospital

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Ochsner and Owens (1) have analyzed the cases of extrathoracic esophagoplasty recorded up to 1933. According to them, the lowest mortality rate and the highest incidence of successful completion of the reconstruction occurred when jejuno-dermato-esophagoplasty was carried out. Their own patient was treated in this manner, and was the first successfully completed case recorded in America. Since then three additional successful reconstructions have been reported by American surgeons (2, 3), and the present case is the fifth to be completed. During this same decade 22 successful cases have been described in Europe and Asia, and in all but 3 of these some form of jejuno-dermato-esophagoplasty was carried out (4–14). There have also been numerous partial reconstructions.

In the past the majority of the patients selected for extrathoracic esophagoplasty have been those suffering from cicatricial atresia of the esophagus resulting from the ingestion of lye or other corrosive substances. Fortunately there has been considerable improvement in recent years in the treatment of fresh chemical burns of the esophagus, so that atresia is not often encountered now. At the same time, great advances have been made in the surgical treatment of esophageal neoplasms, and thus it is to be hoped that an ever increasing number of cured cancer patients will seek the restoration of deglutition.

In nearly every case reported, errors were made and mishaps occurred which could have been avoided had the available information on the subject been used. This was true in our own experience, and this fact together with the probable increase in demand for esophagoplasty has prompted the present communication.

* This case was presented before the Johns Hopkins Medical Society, Nov. 10, 1941.

Case report. L. D., a white girl aged 2 years and 11 months, was admitted to the Johns Hopkins Hospital on October 8, 1928. Three weeks previously she had swallowed a quantity of concentrated lye. The family physician had treated her with a mixture of lemon juice and vaseline, resulting in healing of the ulcerations of mouth and throat,

FIG. 1. FIFTEEN YEAR OLD GIRL. COMPLETE OBLITERATION OF THE ESOPHAGUS FROM NECK TO STOMACH FOLLOWING LYE BURN WHEN THREE YEARS OLD

Gastrostomy tube in place. Note fistula in neck

but there was progressive stenosis of the esophagus. During the last 4 days before entering the hospital immediate and total regurgitation occurred of food or fluids taken by mouth.

Gastrostomy was performed on the day following admission. During the following weeks esophagoscopy was attempted several times, but bougies could not be passed into the stomach, nor could a string

be successfully swallowed. Further evidence of complete cicatricial closure of the esophagus was adduced from the constant regurgitation of saliva. There were repeated attacks of aspiration pneumonia, complicated twice by empyema, and there was no gain in weight for nearly 18 months.

In June, 1930, the cervical portion of the esophagus was exposed, and one month later an external esophageal fistula was made. The effect on the patient was extremely beneficial; there were no further attacks of pneumonia and a rapid increase in weight took place. The external opening of the fistula, which was located just above the medial end of the left clavicle, tended perversely and persistently to close spontaneously. Bouginage and catheterization were ineffectual; and finally a permanent opening was created by turning in and attaching skin flaps to the wall of the esophagus. Further operative procedures were postponed until the patient should have gained in weight and stature, and also because we did not know what effect growth might have upon a reconstructed esophagus.

The patient returned after a number of years. During the intervening period she had been nourished entirely through a gastrostomy opening. The fistula in the neck had remained patent, and there was free discharge of saliva through it.

Following exploration of the stomach to make certain that no useful intrathoracic portion of the esophagus remained, the construction of an extrathoracic skin channel was begun in May, 1940.

A study of the possibilities and methods of forming an extrathoracic esophagus was made, and the decision arrived at was influenced by the fact that there was no slack skin over the anterior portion of the chest wall, and that the breasts were developing perfectly, in a normal position. It seemed that in a 16 year old girl, any unnecessary distortion of the breasts in addition to the strain which she had been enduring through the years would have a bad psychological effect. So it was determined to construct the extrathoracic esophagus by forming a channel of whole thickness skin with its subcutaneous tissue, arranged to include the fistula in the neck, and then to cover the raw surface of this channel and the raw surface made by its formation with other whole thickness skin and subcutaneous tissue brought in by means of tubed flaps.

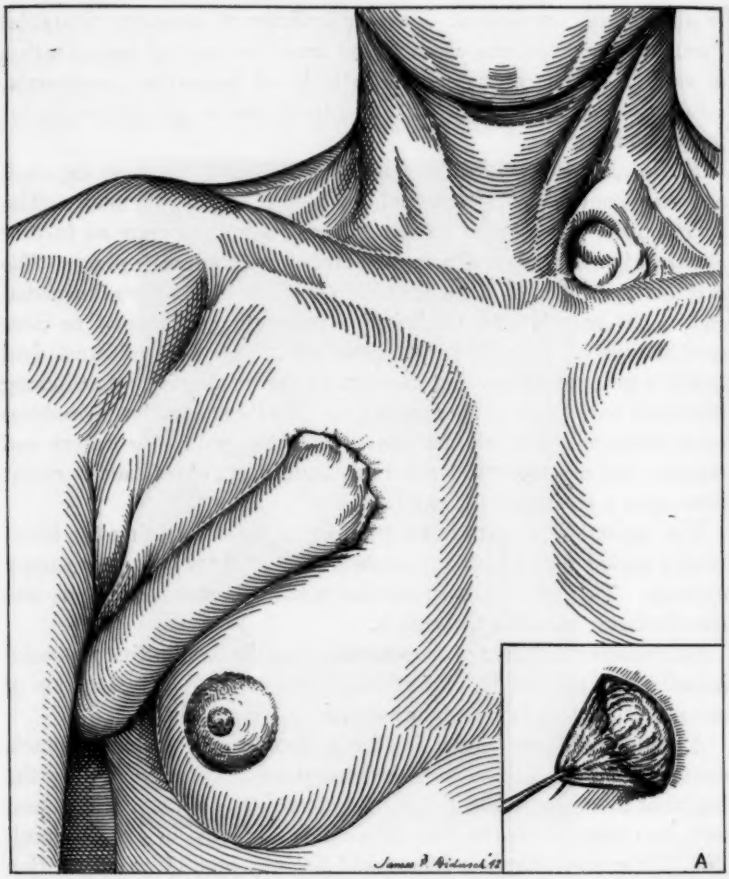

FIG. 2. SCHEMATIC DRAWING SHOWING THE SKIN LINED FISTULA IN THE NECK, WHICH OPENS INTO THE STUMP OF THE ESOPHAGUS

A tubed flap had been formed in the axillary region, its lower pedicle had been divided, and the free end brought toward the midline where it was sutured into a prepared defect as shown in insert.

The first attempt, May 23, 1940, was to make a single long tubed flap which was intended to supply the entire covering for the whole extrathoracic esophagus, but this did not work out as expected because

of several complications. Therefore only a portion of the original long tube was eventually available, and this in the form of a tubed flap after one pedicle had been cut. This loss in length made it necessary

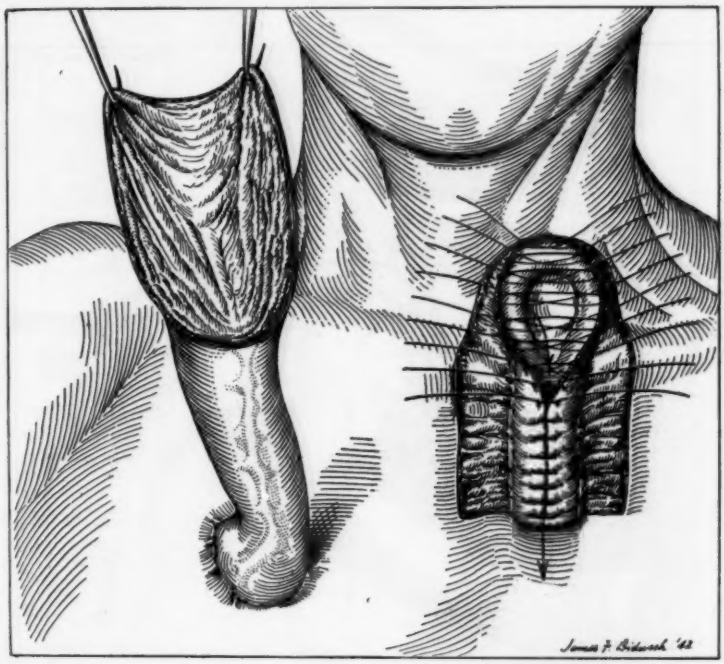

FIG. 3. SCHEMATIC DRAWING SHOWING THE POSITION OF THE INCISION THROUGH THE SKIN AND SUBCUTANEOUS TISSUES INCLUDING THE FISTULA IN THE NECK, AND THE METHOD OF FORMING THE SKIN CHANNEL

The margins inside the incision were drawn together with sutures, thus forming the upper portion of the new skin lined channel. The posterior pedicle of the tubed flap had been divided, all scar tissue removed, and a suitable portion had been opened up.

to revise our procedure to some extent, and while the principle was the same its method of accomplishment was different, as two tubed flaps were required instead of one.

July 18, 1940. The free end of the tube on the right side of the

chest wall was opened for a distance of 3–4 cm. and all scar tissue was excised. The opened end was carried across the chest wall toward the midline as far as its pedicle would allow. At this point a crescentic shaped incision about 4 cm. long with its curve toward the midline was

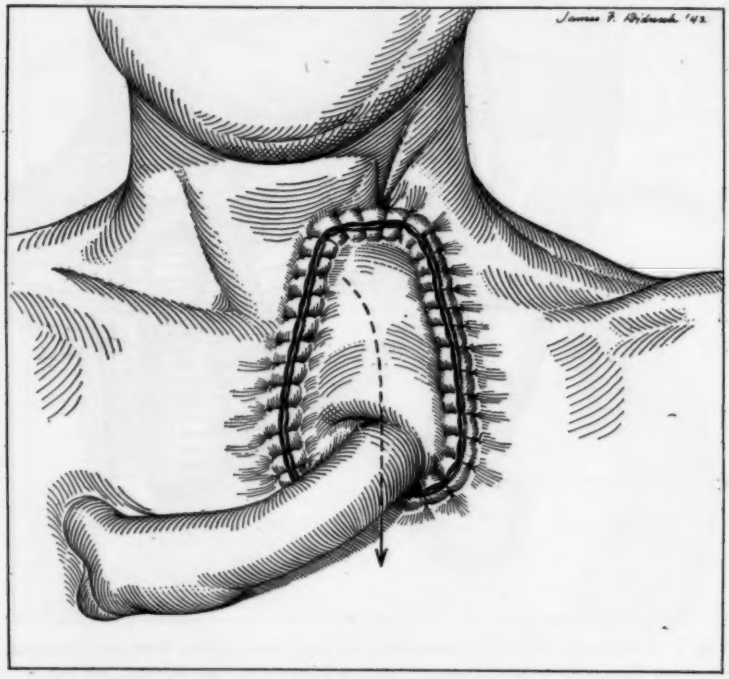

FIG. 4. SCHEMATIC DRAWING SHOWING THE UPPER SECTION OF THE SKIN CHANNEL, AND THE OPENED OUT PORTION OF THE TUBED FLAP PLACED OVER IT

This was sutured into position with "on-end" mattress sutures. Note the direction of the arrow.

made, and the included flap of skin and subcutaneous tissue was turned outward, thus making a more or less circular raw surface. Into this defect, the opened out free end of the tubed flap was sutured carefully. The flap was adequately supported and in due time after healing was complete the patient returned home for two months.

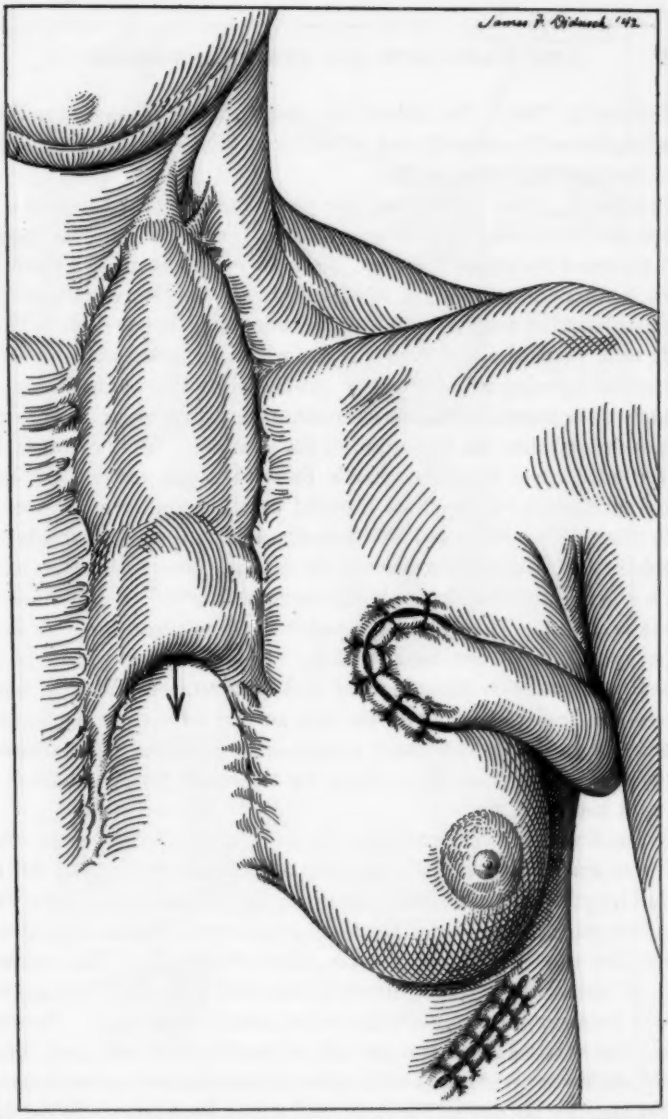

FIG. 5. SCHEMATIC DRAWING SHOWING A SECOND TUBED FLAP, WHICH HAD BEEN FORMED IN THE LEFT AXILLARY REGION AND HAD BEEN BROUGHT FORWARD AS ON THE RIGHT SIDE

Note the length of the partially formed extrathoracic esophagus after the pedicle of the tubed flap on the right side had been opened and used to cover a further extension of the skin channel.

October 1, 1940. The tubed flap which was in excellent condition was lengthened posteriorly and tubed for an additional distance of 7–8 cm., thus adding more length.

October 31, 1940. The posterior pedicle of the tubed flap was divided, and after removing the scar, this portion of the flap was opened and flattened for about 7–8 cm. Then two vertical incisions parallel to each other and about 7 cm. apart and 10 cm. long were made so that the fistula in the neck was midway between their upper ends. These ends were joined by a curved incision which extended above and included the opening into the neck. At the inner side of the lower end of each of the parallel incisions an incision 2 cm. long was made at right angles to facilitate the formation of the channel. After mobilization of the margins of the tissue inside these incisions, leaving the base intact, the edges of skin were brought together and were sutured to each other with interrupted 000 chromic catgut sutures tied inside and supplemented with other sutures in the subcutaneous tissue, thus forming a channel lined with whole thickness skin with its subcutaneous tissue attached. This channel included the fistula into the neck in its upper part and was continuous with it. The opened out portion of the tubed flap was then brought over and was sutured with fine black waxed silk and horsehair into the raw surface over the channel, thus closing the entire defect without tension and making an external covering of skin and subcutaneous tissue for the newly formed channel as far as it had been made.

In due time, the pedicle attached to the anterior portion of the chest wall was separated, and the tubed flap was opened and spread out for its full length. An additional portion of the channel continuous with that first made was formed in the same general way, and the opened out tubed flap was sutured to the skin, thus covering it. This made a channel continuous with the fistula in the neck and about half as long as was necessary to complete the extrathoracic esophagus. Another tubed flap was made on the left side of the thoracic wall and, when ready, its lower pedicle was cut and was sutured into a curved incision on the anterior portion of the chest wall as had been done with the flap on the other side. When conditions were favorable, the posterior pedicle was divided. When the free end of the flap was brought to the midline in anticipation of covering the next section of the channel in

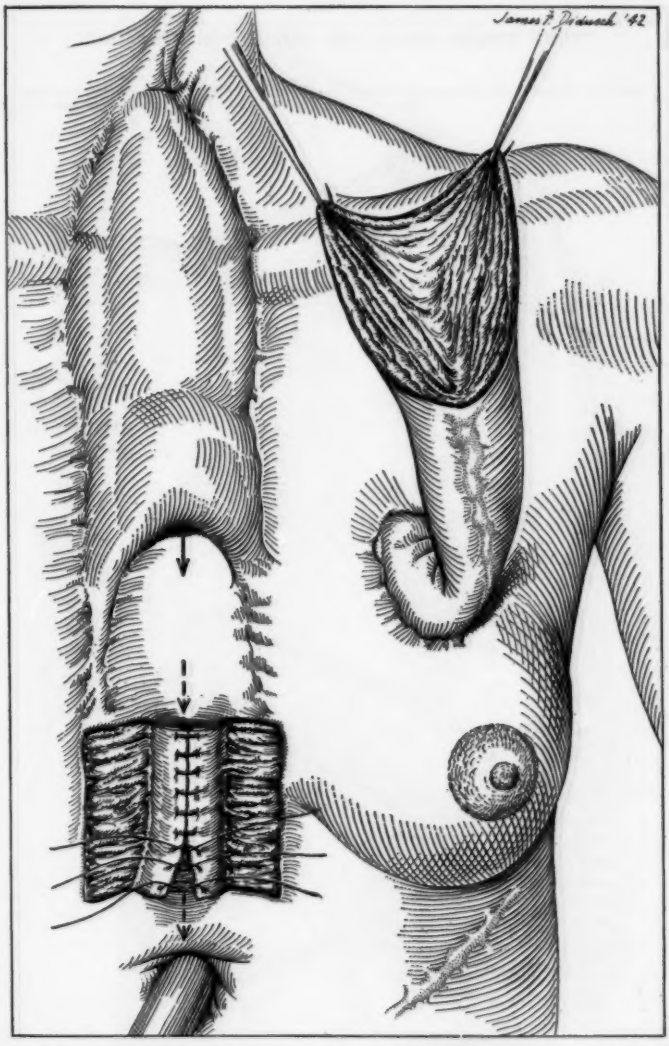

FIG. 6. SCHEMATIC DRAWING SHOWING THE TUBED FLAP ON THE LEFT SIDE OPENED AND SCAR TISSUE REMOVED AFTER SEPARATION OF ITS POSTERIOR PEDICLE

It was found that because of circulatory disturbance, it could not safely be brought over to cover the skin channel if formed in continuity. The lowest portion of the channel was then formed first in the usual way, and the opened out tube was used to cover it.

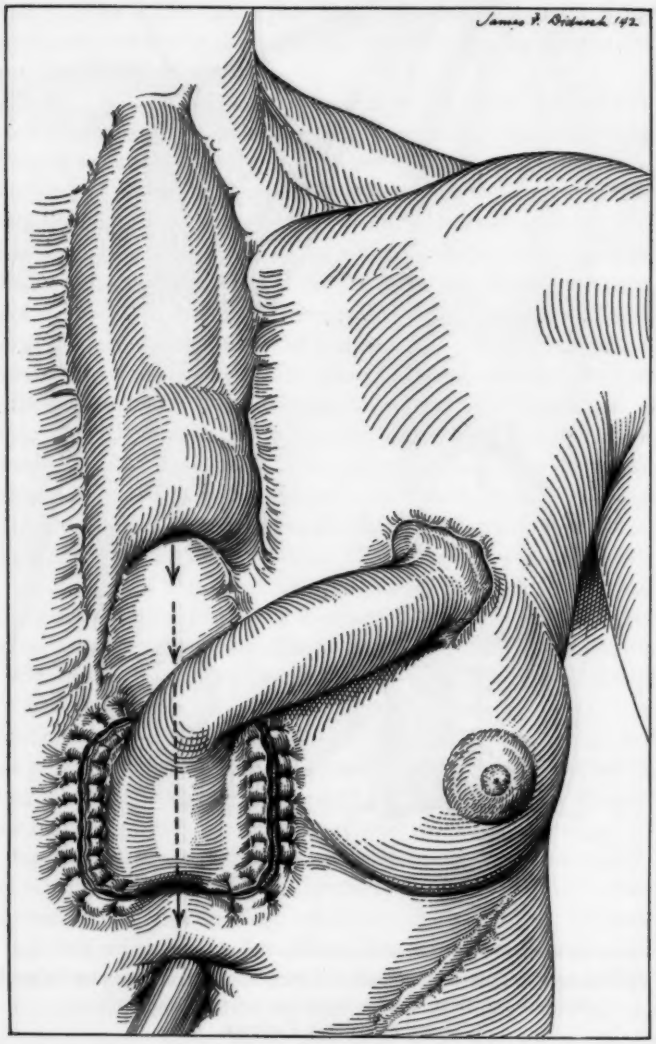

FIG. 7. SCHEMATIC DRAWING SHOWING THE LOWEST PORTION OF THE CHANNEL FORMED AND COVERED WITH THE OPENED OUT PORTION OF THE TUBED FLAP

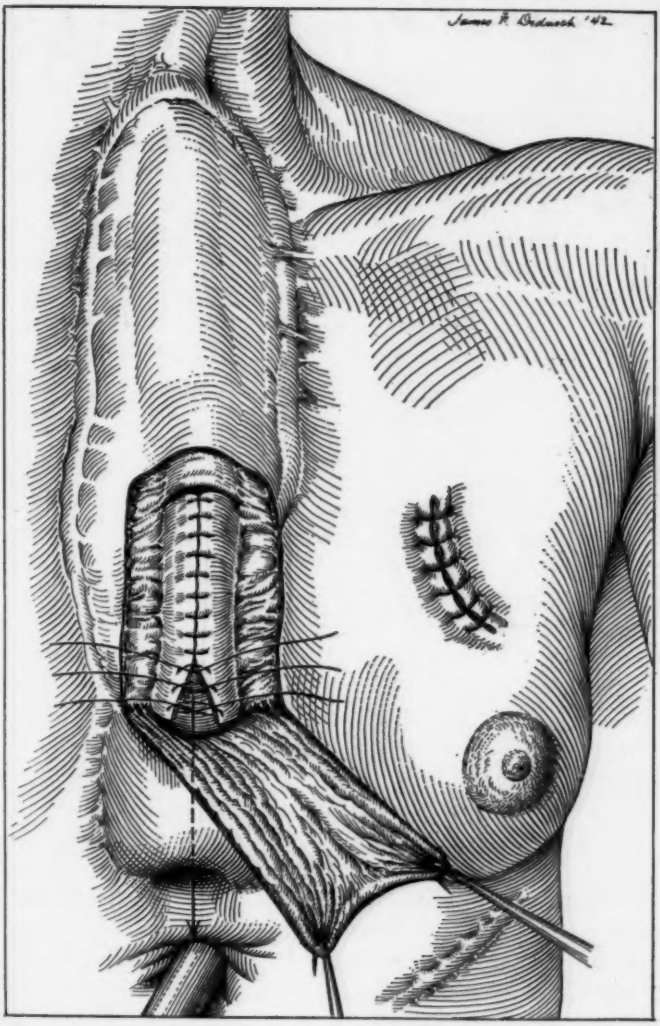

FIG. 8. SCHEMATIC DRAWING SHOWING THE INTERVENING PORTION OF THE SKIN CHANNEL FORMED; THE PEDICLE OF THE TUBED FLAP SEPARATED FROM THE CHEST WALL AND OPENED UP TO COVER THE COMPLETED SKIN FORMED PORTION OF THE EXTRATHORACIC ESOPHAGUS.

Note the postion of the gastrostomy tube

continuity, it was found that its application at that point would interfere with its circulation. For this reason, it became necessary to construct the portion of the channel adjacent to the gastrostomy opening first, and this was done in the usual way. When the pedicle on the chest wall was finally separated, the portion of the channel between the areas previously made was completed by the same type of procedure, and the channel extending from the neck to the costal margin was completely formed. All of these procedures, the last being on May 10, 1941, took about one year to accomplish. Finally, the extrathoracic esophagus was prepared and was ready for its hook-up with the stomach.

This procedure was undertaken on October 2, 1941. The abdomen was entered by means of a left paramedian incision, the upper end of which terminated 4 cm. below the end of the extrathoracic gullet. A segment of jejunum 20 cm. in length was selected for transplantation, this portion commencing about 10 cm. below the ligament of Treitz and having a long, mobile mesentery. Stone intestinal clamps were used, the bowel was divided, and the mesentery was incised nearly to the root, great care being taken to preserve the circulation of the isolated segment. The isolated intestine and its mesentery were placed to the patient's left, and the intestinal continuity was reestablished by means of an aseptically performed end-to-end anastomosis of the upper and lower portions of the jejunum. Two rows of interrupted Lembert sutures of fine silk were used. The defect in the mesentery was closed by approximating the edges with interrupted fine silk sutures.

Openings were then made in the mesocolon and the gastrocolic omentum, well to the left of the midline. The isolated segment of jejunum was brought through these openings into a position in the upper left abdomen adjacent to the cardia of the stomach. Care was taken to prevent rotation of the mesentery of this segment. The clamp was removed from the caudal end of the transplanted jejunum, and an "open" anastomosis was carried out between the anterior wall of the stomach and the end of the bowel. The anastomosis was placed as high as possible on the stomach; its performance was rendered difficult by the fixation of the stomach wall due to the proximity of the gastrostomy. An inner continuous suture of No. 0 chromic catgut was reinforced by an outer row of interrupted Lembert sutures of fine silk.

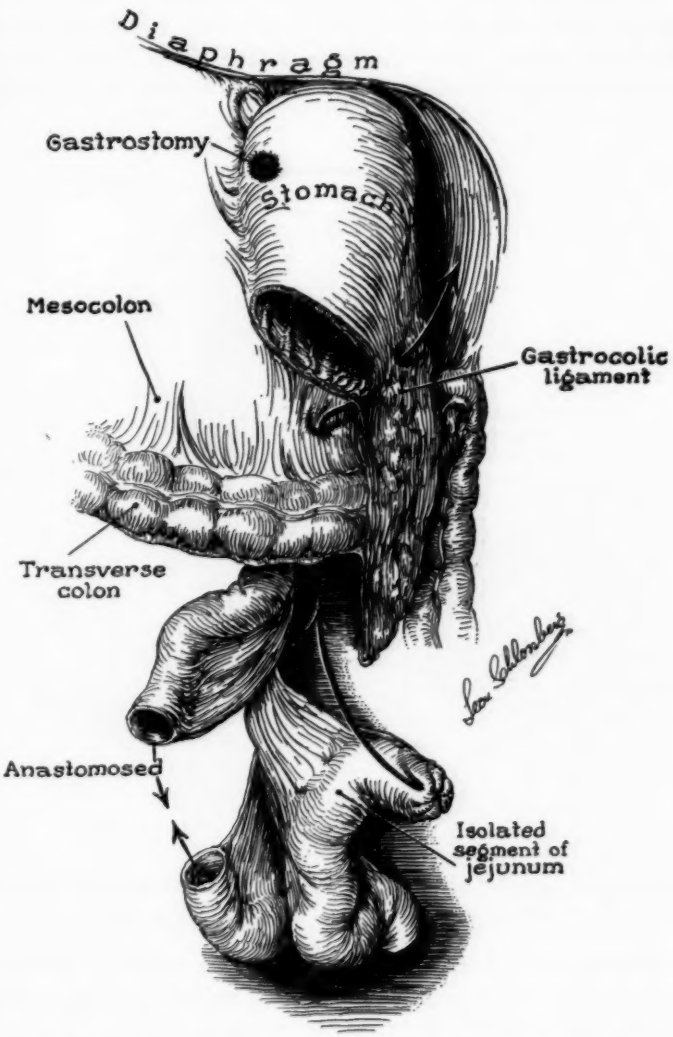

FIG. 9. SCHEMATIC DRAWING SHOWING THE ISOLATION OF THE JEJUNAL SEGMENT

The cephalad end of the jejunal segment was brought out of the abdominal cavity through the upper angle of the abdominal incision. The lower portion of the incision was then closed, interrupted silk sutures being used throughout; but in the upper angle no attempt was

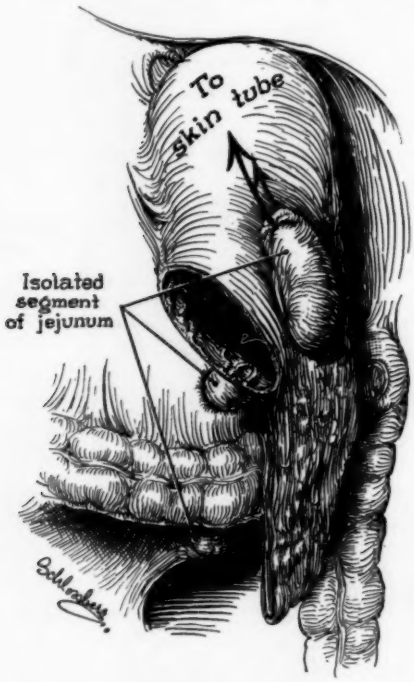

FIG. 10. SCHEMATIC DRAWING

The jejunal segment with attached mesentery is drawn up through openings made in the transverse mesocolon and gastrocolic omentum.

made to approximate the peritoneum or the rectus sheath. It was now apparent that more than sufficient jejunum had been isolated, and that the circulation of the bowel remained excellent.

A tunnel was made under the skin from the upper angle of the abdominal incision to the lower end of the extrathoracic gullet. The end

of the inner skin channel was separated from its bed. The jejunum was passed through the tunnel, and the excess jejunum amputated. Since the circulation of the jejunum was impaired by the compression of the overlying skin, a relaxation incision was made through the skin

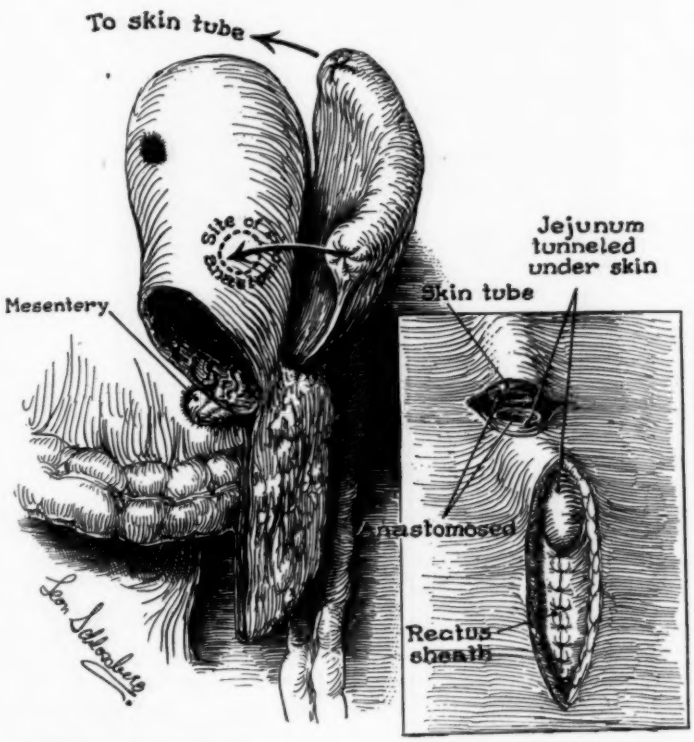

FIG. 11. SCHEMATIC DRAWING SHOWING THE SEGMENT OF JEJUNUM IN ITS FINAL POSITION

of the thoracic wall about 6 cm. lateral to the tunnel. This incision relieved the tension, and the jejunal stump bled freely. The intestine was not put on the stretch, but care was taken to prevent redundancy. The end of the jejunum and the end of the inner skin channel were ap-

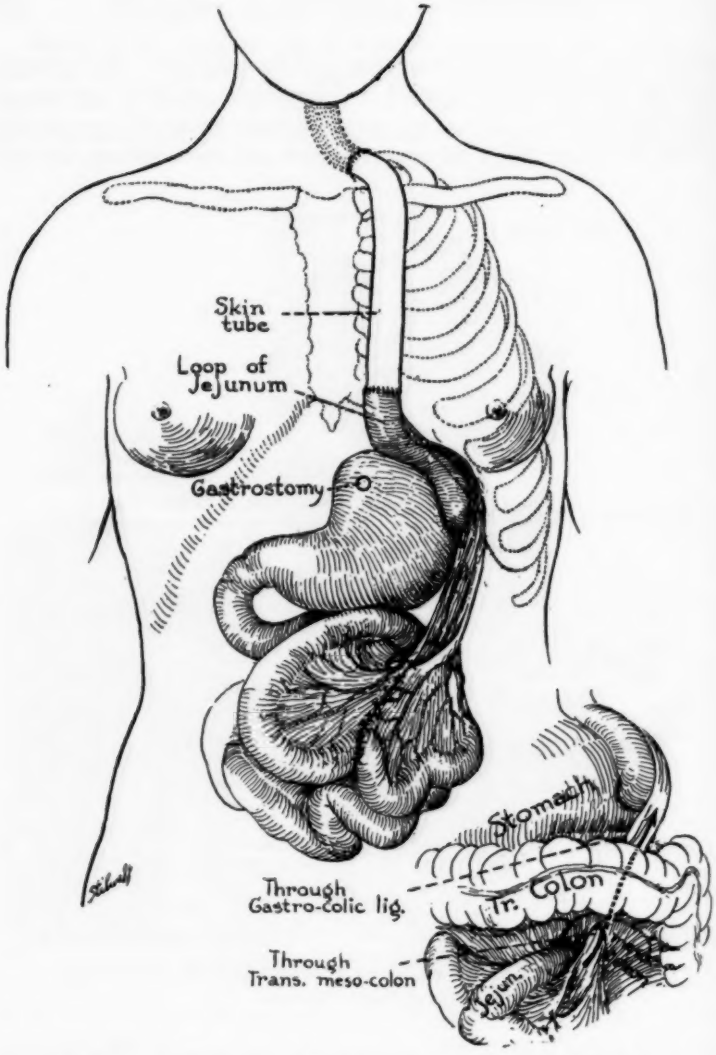

FIG. 12. SEMI-DIAGRAMATIC ILLUSTRATION SHOWING THE COMPLETED RECONSTRUCTION

The transverse colon has been omitted from the large drawing for the sake of clarity.

proximated by means of a single row of interrupted No. 0 chromic catgut end-on mattress sutures.

The edges of the relaxation incision could now be approximated without impairing the circulation, and a small rubber drain was in-

FIG. 13. RESULT THREE MONTHS AFTER FORMATION OF EXTRATHORACIC ESOPHAGUS BY THE METHOD REPORTED IN THIS COMMUNICATION

Note the scars outlining the tubed flaps used in covering the skin lined channel. Note the normal position of the breasts. The gastrostomy opening has contracted considerably but has not closed spontaneously. An operative closure was made six months later. Note the well nourished condition of the patient.

serted into the lower angle. The skin overlying the extrathoracic gullet was approximated to the upper end of the skin channel, and the skin of the abdominal incision also was closed with interrupted silk sutures, completing the reconstruction.

The patient withstood this operation very well, as she had all the others. Feeding through the gastrostomy tube was maintained for the first week, after which time fluids were gradually given by mouth. Within two weeks all feeding by the gastrostomy was discontinued. Solid food, thoroughly chewed, was permitted 21 days after operation, and the patient has been on an unrestricted diet since then. She has been cautioned to chew thoroughly, and to drink fluids after eating solid food.

The abdominal incision and the relaxation incision healed per primam. On the tenth postoperative day two small fistulae developed at the site of the anastomosis between the extrathoracic skin channel and the jejunum. Profiting by the experience of others, we made no attempt at treatment, and these fistulae healed spontaneously at the end of two months. No attempt was made at that time to close the gastrostomy. We felt it would be a safeguard in case of mishap, and that it would eventually close spontaneously. Eight months after the final operation, the gastrostomy had contracted considerably, and there was not much drainage, but inasmuch as the marginal lining of the gastric fistula was shown to be gastric mucosa by biopsy, it was doubtful whether complete spontaneous closure would take place in a reasonable time. In order to expedite the final closure, on June 6, 1942, the margins of the outer end of the fistula were mobilized, and turned in, so that raw surface was sutured to raw surface, and over the defect a flap of adjacent skin was shifted and sutured, thus closing the fistula. This method was selected because of the position of the fistula and on account of the extent of scar involvement of the surrounding tissues.

Studies have been made of the function of the new gullet. Following the act of swallowing, air, fluid, and food pass down the skin channel rapidly and forcefully. Active peristalsis can be observed in the jejunal segment, the contraction waves passing from above downward. The position of the patient has little effect on the passage of ingested material. The patient is at present leading a normal life, is eating with obvious relish and is steadily gaining weight. A roentgenogram (November 25, 1941) of the completed extrathoracic esophagus taken after the ingestion of bismuth shows a well formed channel from neck to stomach with a slight constriction at the point of anastomosis of the jejunal segment.

COMMENT

Such considerations as the wisdom of attempting extrathoracic esophagoplasty, the choice of procedure, the age of the patient, and the manner of handling complications warrant discussion. It should be emphasized that before undertaking reconstruction one must make certain that the intrathoracic esophagus is beyond repair. It is important, therefore, to explore the esophagus from the gastric end as well as from above, since a short atretic segment may be dealt with directly. In our patient bougies introduced orally could not be passed below the level of the suprasternal notch, and on exploring the stomach no trace of the esophageal orifice could be found, even by direct inspection.

There can be little argument as to the desirability of restoring deglutition in a patient who must otherwise face the prospect of being nourished by means of a gastrostomy, especially since there is little operative risk in the procedures entailed. In the case of a patient who has undergone removal of the esophagus for cancer, however, the surgeon is justified in postponing reconstruction until permanent cure seems likely; and in a patient of advanced years the surgeon may hesitate to undertake the series of necessary operations.

As a rule, gastrostomy is performed initially in these patients as a life-saving measure, but even at this time the surgeon may facilitate a future reconstruction by properly locating the gastrostomy opening. In our patient the gastrostomy was placed high in the midline, so that the new gullet had to be detoured around it. A more convenient location would have been some point along the left costal margin lateral to the mammary line. In this connection it is interesting that our patient was nourished entirely through the gastrostomy for over 13 years. Considerable study was made of the feeding problem and a formula was evolved which proved satisfactory and provided for proper growth and development.

Formula for Tube Feeding (24 hour total). 3 eggs, 12 cups evaporated milk, ¼ lb. sieved liver, 5 tablespoons salad oil, ½ cup puréed vegetables, 1½ cups mashed potato, ½ cup molasses, ½ cup puréed prunes, 1 cup cooked oatmeal, ½ cup sugar, ½ cup orange juice, 1 tablespoon brewers' yeast.

Protein, 171 grams; fat, 225 grams; carbohydrate, 547 grams. Total calories, 4897; total fluid, 3000 cc.

Ten years elapsed between the first stage of the reconstruction (creation of a cervical esophageal fistula) and the construction of the extrathoracic gullet because we did not know what effect the growth of the patient might have upon a new esophagus. This time need not have been lost, for Lundblad (6) and Biezins (9) carried out successful reconstructions in patients aged only 2 or 3 years. In Lundblad's patient, when observed at the age of 19, the extrathoracic gullet functioned perfectly. Several other patients have been observed for varying periods, and in only an occasional case has difficulty arisen, usually in the form of a cicatricial stenosis at the point of junction of the skin channel with the jejunum. This complication has been dealt with satisfactorily by means of bouginage.

In our patient the maintenance of the cervical esophageal fistula at first proved to be a troublesome problem. Despite frequent dilatation the opening tended to cicatrize, and it was finally necessary to line the tract with small skin flaps. It seems likely that the method advocated by Ochsner and Owens (1) would be more satisfactory in certain cases, for they prepared the extrathoracic skin channel before exposing the cervical esophagus, and thus could anastomose the two structures at once, without creating an external fistula. If this had been done in our case however, it is likely that the patient would have died from aspiration pneumonia.

In connecting the skin tube to the stomach the superiority of the method which employs a segment of jejunum is unquestioned. Experience has shown that this portion of the gut joins readily and smoothly to either skin or stomach, with the occurrence of true healing. It is doubtful whether gastric mucosa ever unites permanently with skin. Segments of the colon have been used for the purpose of joining skin channel and stomach, but it seems unnecessary to add the hazard of operating upon this bacteria-laden organ when the jejunum will serve as well or better. The development of a jejunal ulcer is not to be dismissed lightly, however, and although not yet reported, will probably occur as frequently in a large series of cases as after other types of gastrojejunal anastomosis. The ultimate fate of the blood supply of the transplanted jejunal segment provides interesting material for speculation. An experimental study of that problem is now being undertaken.

The fistulae which developed after operation in our patient healed spontaneously. In nearly every reported case fistula formation complicated the wound healing, and where steps were taken to close the fistulae the results were not good. Apparently such fistulae as develop will close spontaneously if permitted to do so.

In this patient the construction of the extrathoracic esophagus was completed in 16 months, but this is a longer period of time than is actually needed. With the experience gained it seems possible now that complete reconstruction could be carried out in half that time or less.

We feel that the method for the construction of the extrathoracic skin channel devised by the senior author (J. S. D.) is superior to those previously described. A longer period of time is needed, and several operative procedures are required, but some of these can be carried out under local anesthesia.

The procedure of utilizing tubed flaps has the advantage of making a thick covering to the newly formed esophagus with an assured circulation; it is much more stable and more assured in its taking than any free graft could be. The opened out tubed flap is applied and sutured into place without tension and therefore without the marked pressure on the newly formed channel, which always occurs when there is undercutting and advancing of the adjacent skin margins with closure over the channel. The suture lines are not superimposed, so if there is a separation, or local infection, it does not necessarily interfere with the newly formed esophageal channel. The avoidance of cosmetic deformity of the anterior chest wall with distortion of the breasts is advantageous. There is also lessened likelihood of future cicatricial stenosis since there is rapid healing with minimal scar tissue formation. The anastomosis of the skin channel with the jejunum is facilitated because of its thick covering and the ease of separation of its lower end.

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COMPARATIVE ACTIVITY OF SULFONAMIDES AGAINST COLIFORM BACTERIA IN THE INTESTINES OF MICE[1]

HAROLD J. WHITE

Department of Pharmacology and Experimental Therapeutics, The Johns Hopkins University and Department of Bacteriology, The Johns Hopkins University School of Hygiene and Public Health

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Investigation of the usefulness of drugs as chemotherapeutic agents in bacterial infections of the intestinal tract is hampered by the fact that natural intestinal infections of man are difficult, if not impossible, to reproduce experimentally. Thus, no direct experimental assessment of the therapeutic value of drugs as agents for use in bacillary dysentery, cholera and the early stages of typhoid fever can be obtained at present. *In vitro* tests of the bactericidal activity of drugs are alone no guide to therapeutic value because of the very complex conditions under which a drug must act in the intestinal tract. For these reasons, a useful index for the therapeutic trial of a drug in intestinal infections would seem to be its bactericidal activity against the normal intestinal flora, particularly the coliform group, of experimental animals.

Several reports (1, 2, 3, 4, 5, 6, 7) have been published on the antibacterial activity of drugs in the intestines of experimental animals. In all of these investigations, coliform densities in stool samples were determined by direct plating methods in differential media, such as desoxycholate agar. A reduction in numbers of coliform bacteria has been taken as the criterion of drug activity. Although a bacterial count based on the numbers of colonies developing in agar plates, may provide a relatively precise measurement of the density of viable organisms in a stool suspension, the amount of labor and material required for plating quantitative dilutions of large numbers of samples definitely limits the usefulness of this method. Another disadvantage of the differential plating technic, as used for coliform counts of stool suspensions, has been stressed by Poth and his coworkers (7). These investigators pointed out the difficulty of differentiating coliform col-

[1] This investigation has been aided by grants from The John and Mary R. Markle Foundation.

onies from colonies of the fecal *Aerobacter* group of organisms in desoxycholate agar. Since the latter group of bacteria was found to be more resistant to drug treatment than the former, and since the coliform count was the index of activity, considerable confusion could be avoided only by further differential studies.

The present study was carried out: 1) to devise a simple and reliable method for measuring the effectiveness of drugs against coliform bacteria in the intestines of mice; 2) to obtain, with such a method, a qualitative comparison of the activity of certain representative sulfonamides, including all members of this group of drugs which are at present being widely used clinically; 3) to investigate the possibility of obtaining quantitative comparisons of the anticoliform activity of drugs in mice.

EXPERIMENTAL METHODS

A preliminary logarithmic coliform count was made on a stool suspension from each of a group of normal mice. The mice were then arranged in groups of five, on the basis of these pre-treatment counts, in such a manner as to obtain approximate equality of average coliform count per group. One group was kept on normal control diet, without drug. The other groups were placed on drug-diets for five days. Measurements of drug-diet intake were made for the period of treatment. A post-treatment coliform count was then made on each mouse. The effect of a drug was expressed in terms of the difference between the pre-treatment and post-treatment coliform counts.

Mice. White male mice (CFCW strain) of 17 to 23 grams weight and from 8 to 10 weeks of age were used throughout this study. The experimental unit consisted of 5 mice.

LOGARITHMIC DILUTION COUNT OF COLIFORM BACTERIA: 1) *Collection of Stool Sample.* Mice were held in an inverted position and the abdominal surface was gently stroked to cause evacuation of fecal pellets. Two pellets from each mouse were allowed to drop into a sterile one-ounce bottle containing 1.0 cc. of sterile tap water. The collecting bottles were then re-plugged with sterile cotton.

2) *Preparation of Filtered Stool Suspension.* The formed pellets were broken up with a small steel spatula which had been dipped in alcohol, flamed and then cooled by immersion in sterile tap water. After the addition of 9.0 cc. of sterile tap water, the stool suspension was thoroughly mixed by means of an electric stirrer the blade of which was sterilized with alcohol as described above. The contents of the bottle were then transferred to a sterile cotton-plugged test-tube (150 x 16 mm.). By means of sterile forceps, a small pad (20 x 20 x 10 mm.) of sterile absorbent cotton was placed in the tube. Filtration was accomplished

by forcing the cotton pad down through the fluid. In this manner, gross particles were filtered out and a uniform suspension of fine stool particles was obtained.

3) *Dilution and Culture of Filtered Stool Suspension.* Successive ten-fold dilutions in broth, out to 10^{-10}, were made as follows: 1.0 cc. of filtered stool suspension was pipetted into a tube containing 9.0 cc. of lactose broth (described below). Thorough mixing of stool and broth was obtained by vigorous rotation of the tube. At this point, the first dilution, i.e., 10^{-1}, consisted of a stool-broth mixture with a total volume of 10 cc. containing 1.0 cc. of the original stool suspension. One cc. of this dilution was then pipetted into a second tube of broth to obtain a 10^{-2} dilution. Thus, the final volume of mixture in the 10^{-1} dilution tube was 9.0 cc. containing 0.9 cc. of the original stool suspension. This procedure of pipetting and mixing was continued through ten dilutions. The successive dilutions resulted in a series of stool-broth mixtures in which the total content of original stool suspension decreased uniformly, by multiples of one-tenth, from 9×10^{-1} cc. down to 9×10^{-10} cc. In this manner, the same medium and tubes were utilized for both dilution and culture of the stool suspension. The following tabulation shows the relation between the degree of dilution and the amount of stool suspension which was cultured in each tube:

TUBE NUMBER	STOOL SUSPENSION	
	Dilution	Amount cultured
		cc.
1	10^{-1}	9×10^{-1}
2	10^{-2}	9×10^{-2}
3	10^{-3}	9×10^{-3}
4	10^{-4}	9×10^{-4}
5	10^{-5}	9×10^{-5}
6	10^{-6}	9×10^{-6}
7	10^{-7}	9×10^{-7}
8	10^{-8}	9×10^{-8}
9	10^{-9}	9×10^{-9}
10	10^{-10}	9×10^{-10}

4) *Dilution Count.* The presumptive count of coliform bacteria was based on the last tube in the dilution series which showed fermentation of lactose with gas. This count was expressed as the common logarithm of the number of coliform bacteria (i.e., the "logarithmic" coliform count) per 9.0 cc. of original stool suspension. For example, if gas production was positive in all tubes up to and including the sixth (i.e., gas production from culture of 9×10^{-6} cc. of stool suspension) the logarithmic count was taken to be 6, or in terms of numbers of bacteria, 1,000,000, coliform bacteria per 9.0 cc. of undiluted stool suspension. It was realized that where gas production changed from positive to negative the number of coliform bacteria responsible for growth in the positive tube may have

varied from one to nine, the most probable number being about 2.3 (8). However, for convenience in recording comparative counts, it was assumed in the present study that gas production in the last positive tube was due to the minimal number of bacteria (i.e., one). Thus, a logarithmic count of 6 represented a probable count of something between 6 and 7, but not less than 6 and not as much as 7. Although a yardstick with such coarse divisions could not be used to measure coliform densities with precision, it was considered to be satisfactory for determining reductions of from 100 to 100,000-fold in the coliform content of stools from mice treated with active drugs.

In culture series which were completely negative, in terms of gas production, the logarithmic coliform count per 9.0 cc. of stool suspension was recorded as zero. Thus, no distinction was made between complete absence of coliform bacteria and the presence of one organism per 9.0 cc. of suspension. This was done because results were recorded and analyzed as logarithmic values. Here again, the fact that the present study was concerned only with very large differences in number of coliform bacteria made the distinction between reduction to sterility and reduction to a single bacterium unimportant.

Coliform counts were confirmed, in all cases, by subculture from all positive gas tubes to Levine's Eosin Methylene Blue agar.

5) *Lactose Broth.* The medium used for dilution and culture of stool suspensions was a buffered Tryptose-lactose broth of the following composition:

Tryptose peptone (Difco)	15.0 grams
Lactose	3.0 grams
Sodium chloride	5.0 grams
Phosphate buffer solution[2]	60.0 cc.
p-Aminobenzoic acid	10.0 mgms.
Distilled water	1000.0 cc.

Heated at 100°C. for 5 minutes to dissolve. Dispensed in tubes (150 x 16 mm.) containing an inverted vial (50 x 5 mm.). After autoclaving at 110°C. for 10 minutes, the volume of broth, exclusive of the contents of the vial, was 9.0 ± 0.1 cc. (pH, 7.2).

6) *Incubation.* The lactose broth cultures were incubated in a water bath at a temperature of 44 ± 0.005 degrees Centigrade. Readings of growth and gas production were made after 48 hours incubation.

STANDARDIZATION OF TEST GROUPS OF MICE. Before each experiment, preliminary logarithmic counts were made on a large group of normal mice. Occasional mice which showed counts of less than 3 were discarded. The remainder were arranged in five classes as follows: 1) counts of 3 and 4; 2) counts of 5 and 6; 3) counts of 7 and 8; 4) counts of 8 and 9; 5) counts of 9 and 10. A mouse from each of these five classes was taken at random to form a test group. Each

[2] 143 grams of disodium phosphate and 14 grams of sodium dihydrogen phosphate dissolved in distilled water to make one liter of solution.

test group thus had approximately the same average coliform count. For example, forty mice were arranged in classes as follows: eight mice with counts of 9–10; eight with counts of 7–8; eight more with counts of 7–8; eight with counts of 5–6; and eight with counts of 3–4. From this distribution, eight test groups, each similarly composed of five mice were formed: one mouse having a count of 9–10; two mice, counts of 7–8 each; one, a count of 5–6, and one, a count of 3–4. Thus, each of the eight test groups had an average logarithmic coliform count of about 6.7 per mouse. Seven of these groups were placed on drug-diets, one group being kept, as a control, on normal diet without drug. In this way, seven drugs could be tested simultaneously for activity against coliform bacteria in the intestines of mice under more or less standardized experimental conditions.

DRUG-DIET TREATMENT. The drug-diet method of treatment (9, 10), in which the drug is self-administered, was used throughout the present investigation. Marshall and his coworkers have shown that under these conditions mice eat with sufficient regularity and frequency to insure the continuous presence of drug in the intestine.

Treatment was carried out by permitting the mice to feed, at will, upon food in which drug was incorporated. The basic diet was Wayne Fox Chow. Drug-diets consisted of finely powdered drug and food intimately mixed in the proportions desired. Occasional analyses of samples taken at random from experimental diets made in this laboratory have shown that the actual drug concentrations were within ±5 per cent of the expected values.

Each mouse was placed in a small individual cage with a food cup containing sufficient drug-diet for five days treatment. The average drug intake in milligrams per mouse per day was computed from food cup weights taken at the beginning and at the end of the treatment period.

DETERMINATION OF DRUG CONCENTRATION IN STOOL SUSPENSIONS. Average values for the sulfonamide content of stool suspensions were usually obtained as follows: equal portions of the five filtered stool suspensions from the individual mice on each drug-diet were pooled and diluted 1:20 with four parts of 15 per cent trichloroacetic acid and fifteen parts of distilled water. After filtration, the drug analysis of the filtrate was carried out by Marshall's method (11). In some cases, an initial dilution of 1:30 was made by adding six parts of acid and twenty-three parts of water to one part of stool suspension. All stool concentrations represented free, not total, drug and were expressed in milligrams per 100 cc. of filtered suspension. By use of appropriate factors, these values could then be converted into approximations of the drug concentration in terms of milligrams per 100 grams of dry or wet stool, or in milligrams per 100 cc. of the stool water content. It was found that there were about 110 milligrams of dry stool per 100 cc. of stool suspension. It was also found that the ratio of dry stool to stool water content was 1:1.5. This meant that, on the average, 100 cc. of filtered stool suspension (110 mgm. of dry stool) was equivalent to about 0.17 cc. (165 mgm.) of stool water. Thus, the concentration of drug as determined in milligrams per 100 cc. of filtered stool suspension, could be converted into milli-

grams per 100 cc. of stool water content by dividing the former value by 0.17 and then multiplying by 100. For example: a concentration of 0.3 mgm. of drug in 100 cc. of suspension would be equivalent to about 180 mgm. per 100 cc. of the water content of the original stool sample.

RESULTS

I. Experimental basis of a method for measuring the anticoliform activity of sulfonamide drugs in the intestines of mice. It was assumed that counts of coliform bacteria obtained on stool samples (fecal pellets) were indirect measurements of the densities of these bacteria in the intestinal contents. Factors, other than specific drug treatment, which may have influenced these counts included the following: the culture medium in which counts were made; incubation temperature; incubation time; *in vitro* effect of drug in stool sample; time required to prepare stool samples for culture; pH of stool samples; reproducibility of counts; undissolved portion of drug in the intestinal contents. In preliminary experiments, the influence of each of these non-specific factors was investigated and, where necessary, controlled, in order to provide a basis on which valid comparisons of specific sulfonamide activity could be made.

It was found that reproducible counts of coliform bacteria could be obtained when stool samples were cultured for 48 hours at 44°C. in a buffered lactose broth containing 1 mgm. per cent of p-aminobenzoic acid. It was also found that a significant reduction in the coliform count of stool samples from treated mice reflected antibacterial action of a drug *in vivo* and not merely an *in vitro* inhibition due to drug carried over into cultures. No abnormal effect of drugs on the pH of stool samples was observed. In a series of experiments in which mice were treated with a diet containing 1 per cent of bismuth sub-carbonate, no anticoliform activity due to mechanical effect of undissolved drug was observed. Detailed results of these preliminary experiments are given elsewhere[3].

Variation of Stool Samples. In the present investigation, all logarithmic counts refer to numbers of coliform bacteria per 9 cc. of a standard stool suspension which was made by mixing two fecal pellets

[3] Experimental data are given in complete detail in the Sc. D. dissertation of the writer in the William H. Welch Library of The Johns Hopkins University.

with 10 cc. of water. These pellets did not appear to vary markedly in size, consistency or frequency whether obtained from mice on the different sulfonamide drug-diets or from mice on the control diet. Thus, the average number of pellets per mouse per day was 135, with a standard deviation of ± 17, based on collections, over a four day period, from 30 mice on different drug-diets and 5 mice on control diet. The average wet weight per pellet was found to be 28.6 ± 7.2 milligrams, based on 80 pellets collected from 35 mice on drug-diets and 5 mice on control diet. The dry weight of these pellets averaged 11.3 ± 2.5 milligrams. The average moisture content, with its standard deviation, was 60 ± 6.0 per cent. Before filtration, therefore, stool suspensions contained about 50 milligrams of wet feces per 9 cc. (about 20 milligrams per 9 cc. on a dry weight basis). After filtration of the stool suspensions, it was found that the amount of stool contained in the filtrate averaged 10.2 ± 2.5 milligrams (dry weight) per 9 cc., based on 195 suspensions of pellets from mice on drug-diets and on control diet. On the basis of a water content of 60 per cent, this average value for the dry stool content of 9 cc. of filtered suspension was equivalent to 25 milligrams of wet stool.

Variation in Coliform Counts on Normal Mice. Before the significance of a reduced coliform count in treated mice could be evaluated, it was essential to obtain information concerning the incidence and normal variation of coliform bacteria in untreated mice.

In preliminary experiments, when mice were taken from stock, at random, it was noted that their logarithmic coliform counts usually ranged from 3 to 9, per 9 cc. of stool suspension. It was also noted that the stool of an occasional mouse apparently contained no coliform bacteria. Under these conditions, the investigation of anticoliform activity of drugs in mice was necessarily based on the use of test groups to which mice were assigned in such a manner as to insure that the groups were random samples and representative of the coliform content of the mice. If the mice had been assigned to groups for testing drugs, wholly at random, there would have been too much chance that low count mice might have got into one of the groups with no compensating high ones. An attempt was made, therefore, to form representative as well as random test groups as follows: mice with logarithmic coliform counts of less than 3 were discarded; mice with

counts of 3, or more, were divided into 5 outcome groups, each composed of animals with similar counts; to each test group one mouse from every outcome group was assigned by random selection. In this way, a balance of high and low pre-treatment coliform counts in the test groups was insured. Every mouse had the same chance of being assigned to a drug-diet, so that any possibility of bias was eliminated.

Pre-treatment counts were obtained on a total of 360 stock mice. Coliform bacteria appeared to be absent in only 6 (1.7 %) of these mice. Individual logarithmic counts on the remainder ranged from 3 to 9, with a mean of 6.01 and a standard deviation of $\pm$ 1.78.

In a series of experiments in which a qualitative comparison of drug activity was obtained, eleven drugs were tested; each drug in four groups of 5 mice each. Eight groups, of 5 mice each, served as con-

TABLE 1

Analysis of variance of pre-treatment logarithmic coliform counts of 260 mice in 13 groups of 20 mice each

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE
Total	259	614.55	
Between group means	12	35.85	2.99
Individual mice within groups	247	578.70	2.34

Differences between group means do not appear to be significant, since the F value of 2.99/2.34, or 1.28, is less than the 5 per cent point, 1.80, for 12 and 200 degrees of freedom.

trols. Drug activity was based on significant reductions in coliform counts after drug-diet treatment for a 5 day period. In these experiments, the initial, or pre-treatment, counts may be considered from the following standpoints: 1) 260 counts on individual mice; 2) 52 mean values for test groups of 5 mice each; 3) 13 mean values for eleven treated and two untreated groups of 20 mice each. The individual pre-treatment counts ranged from 3 to 9, with a mean of 6.33 and a standard deviation of $\pm$ 1.53; test group means ranged from 4.6 to 7.4, with a standard deviation of $\pm$ 0.68; mean values for mice grouped according to treatment ranged from 5.80 to 6.95, with a standard deviation of $\pm$ 0.39. In table 1 is an analysis of the variance of these pre-treatment counts which indicates that the test groups had been standardized in a satisfactory manner, i.e., the pre-treatment

coliform contents of test groups were homogeneous. It is also evident, from this analysis, that the eleven groups of 20 mice each, in which drugs were tested, were all similarly composed with respect to initial coliform content.

Given similar pre-treatment coliform concentrations to act upon, drugs were considered to be active only when they brought about a change which was significantly greater than the change in untreated mice under the same experimental conditions. Differences between the initial and final counts (corresponding to pre- and post-treatment counts, respectively) in 40 untreated mice are given in table 2. These results show: changes in the counts of the 40 individual mice ranged from an increase of 4.0 to a decrease of 4.0, with a standard deviation of $\pm$ 2.04; changes in mean values for the 8 test groups, of 5 mice each, ranged from an increase of 1.2 to a decrease of 1.4, with a standard deviation of $\pm$ 0.88; the average change in count was an increase of 0.02, with a standard error of $\pm$ 0.32. It is evident from these data that the final coliform counts of the 40 untreated mice, considered as a whole, did not differ significantly from the counts which were made on these mice at the beginning of the 5 day experimental period; i.e., the initial and final counts of these mice, as a whole, were homogeneous.

The experiments described above provided information concerning four sources of variation in the coliform counts of normal mice: 1) in stock mice taken at random; 2) in pre-treatment counts of mice in standardized test groups; 3) in final counts of control mice kept under test conditions; 4) in differences between initial and final counts of untreated mice. Information concerning variation, from day to day, in the coliform counts of normal mice was obtained in the following experiments. Five mice, each with a coliform count of 0, were selected from stock. This group of negative count mice, together with a group of five mice with counts of 3 and a similar group with counts of 6, were kept on the basic diet, under test condition, for a period of 10 days. Coliform counts were obtained daily, from the 6th to 10th day, on each of the 15 mice. The results of this experiment are shown in table 3. These data show that considerable variation (up to 10,000-fold) occurred from day to day in the counts of individual mice in both the high and medium count groups. However, it should be noted that the average values for each group varied less than 100-fold, due to

TABLE 2

Reduction of logarithmic count of coliform bacteria in feces of mice kept under test conditions on control diet for 5 days

MOUSE		FOOD INTAKE	LOGARITHMIC COLIFORM COUNT PER 9 CC. OF STOOL SUSPENSION		
Group	Number		Initial	Final	Reduction
		gm./day			
1	1	4.6	7	5	2
	2	5.2	7	7	0
	3	4.8	7	6	1
	4	4.6	5	8	−3
	5	3.8	5	5	0
Average		4.6	6.2	6.2	0.0
2	6	4.0	7	6	1
	7	4.1	7	6	1
	8	3.0	3	7	−4
	9	3.7	3	6	−3
	10	4.0	3	3	0
Average		3.8	4.6	5.6	−1.0
3	11	5.7	7	7	0
	12	5.4	7	7	0
	13	4.1	5	7	−2
	14	3.9	5	6	−1
	15	5.1	5	4	1
Average		4.8	5.8	6.2	−0.4
4	16	4.8	6	8	−2
	17	5.6	6	5	1
	18	4.6	6	3	3
	19	5.8	6	5	1
	20	4.8	6	8	−2
Average		5.1	6.0	5.8	0.2
5	21	4.7	7	5	2
	22	4.0	7	3	4
	23	3.3	7	8	−1
	24	4.4	6	8	−2
	25	4.9	3	4	−1
Average		4.3	6.0	5.6	0.4

TABLE 2—*Concluded*

MOUSE		FOOD INTAKE	LOGARITHMIC COLIFORM COUNT PER 9 CC. OF STOOL SUSPENSION		
Group	Number		Initial	Final	Reduction
		gm./day			
6	26	4.9	8	5	3
	27	4.4	8	5	3
	28	4.6	8	5	3
	29	5.1	7	9	−2
	30	4.6	3	3	0
Average		4.7	6.8	5.4	1.4
7	31	5.7	9	7	2
	32	4.7	7	7	0
	33	4.3	7	5	2
	34	4.4	7	6	1
	35	4.5	5	8	−3
Average		4.7	7.0	6.6	0.4
8	36	4.3	9	9	0
	37	5.4	7	6	1
	38	5.5	7	8	−1
	39	3.6	7	9	−2
	40	4.6	5	9	−4
Average		4.7	7.0	8.2	−1.2
Mean (of 40 mice)		4.59	6.18	6.20	−0.02

increases being balanced by decreases, within groups. It was found that the five mice with coliform-negative stools continued to yield negative counts throughout the test period. In another experiment, five mice with an average coliform count of 5.0 were maintained on normal diet, under test conditions, for a period of 20 days. Counts made on each of these mice, on the 10th, 15th, and 20th days, averaged 4.4, 4.8 and 4.6 respectively.

Thus, it was evident that if logarithmic counts were taken as average values for groups of 5 mice, one could expect these average values to change not more than 100-fold, provided that the mice were kept on the basic diet, under test conditions. On this basis, it was assumed

that a change of more than 100-fold in the average count of 5 mice on a drug-diet could be taken as an indication of drug activity.

Duration of Optimum Treatment. The following experiment was carried out to determine, approximately, the number of days of drug diet treatment required for optimal reduction of coliform count. Two groups, of five mice each, with average pre-treatment counts of 5.8, were placed on 1 per cent drug-diets. One group was given sulfathiazole; the other, sulfaguanidine. Coliform counts were made on each of the ten mice after 2, 5, 8, and 13 days treatment.

TABLE 3

Variation from day to day in coliform count of mice on normal diet

MOUSE NUMBER	LOGARITHMIC COLIFORM COUNTS PER 9 CC. OF STOOL SUSPENSION					
	Days					
	0	1	2	3	4	5
1	7	8	6	8	5	7
2	5	6	6	5	4	3
3	4	5	5	3	4	6
4	4	4	5	8	7	4
5	4	3	4	5	6	5
Average.....	4.8	5.2	5.2	5.8	5.2	5.0
6	4	4	5	4	3	3
7	4	4	4	3	5	4
8	4	3	5	3	4	6
9	3	3	4	5	6	5
10	3	3	4	5	5	4
Average.....	3.6	3.4	4.4	4.0	4.6	4.4

The results are given in table 4. These data indicate that: 1) treatment for two days was not sufficient to produce the maximum reduction in coliform count; 2) the reduction obtained after five days was not significantly enhanced by continuing the treatment up to thirteen days. Although treatment for three or four days might have been as effective as five days, the latter period of time was found to be most convenient, in the present study, for the routine testing of drugs.

In another experiment, a group of ten mice with an average pre-treatment count of 6.6 were given 1 per cent sulfaguanidine drug-diet

treatment for 5 days. At the end of the fifth day of treatment the average coliform count of these mice had dropped to 2.7. The mice were then taken off the drug-diet and placed on normal diet. Coliform counts obtained on the fifth and tenth days after return to normal diet averaged 4.9 and 6.2 respectively. Thus, although the usual reduction in count of about 10,000-fold was brought about by treatment with sulfaguanidine, the effect did not last long after treatment was stopped; the coliform count gradually increased to its pre-treatment value.

II. ANTICOLIFORM ACTIVITY OF SULFONAMIDES. The experiments described above established a basis for a method of determining the activity of drugs against coliform bacteria in the intestines of mice. This method was used in a series of tests to determine the activity of

TABLE 4

Duration of optimum treatment

1 PER CENT DRUG-DIET	LOGARITHMIC COLIFORM COUNT PER 9 CC. OF STOOL SUSPENSION AFTER TREATMENT FOR				
	Days				
	0	2	5	8	13
Sulfathiazole	5.8	3.8	0.4	0.4	0.6
Sulfaguanidine	5.8	4.6	2.0	2.4	1.8

Figures above are average values based on five mice treated with each drug-diet.

ten sulfonamide compounds, each of which was fed in a concentration of 1 per cent in the diet to 20 mice. These compounds included: the parent compound, sulfanilamide (4-aminobenzene-sulfonamide); five sulfonamides which have been released by the Federal Food and Drug Administration, namely, sulfapyridine [2-(4′-aminobenzenesulfonamido)pyridine], sulfathiazole [2-(4′-aminobenzenesulfonamido)thiazole,] sulfaguanidine [(4-aminobenzenesulfonyl)guanidine], sulfadiazine [2-(4′-aminobenzenesulfonamido)pyrimidine] and N^1acetylsulfanilamide [N-(4-aminobenzenesulfonyl)acetamide]; the recently introduced sulfapyrazine [2-(4′-aminobenzenesulfonamido)pyrazine], which has been reported as highly active against bacterial infections (12); N^1 benzoylsulfanilamide [N-(4-aminobenzenesulfonyl)benzamide] and N^4 succinylsulfathiazole [2-(4′-succinylaminobenzenesulfonamido)thiazole], both

of which have been reported to be of interest for use in intestinal chemotherapy (4, 5, 7); and sulfanilylarsanilic acid [4-(4′-aminobenzenesulfonamido) phenylarsonic acid], a sulfonamide which is of interest because of its limited absorption from the intestinal tract.

The sulfanilamide used in this investigation was obtained from Eli Lilly and Company; the sulfapyridine, sulfaguanidine, sulfadiazine and N^1 benzoylsulfanilamide from the American Cyanamid Company; the sulfathiazole from E. R. Squibb and Sons; the sulfapyrazine from Mead Johnson and Company; and the N^1 acetylsulfanilamide from the Schering Corporation. The sulfanilylarsanilic acid and succinylsulfathiazole were prepared in this laboratory by Dr. R. G. Shepherd. The writer wishes to thank these companies and Dr. Shepherd for their courtesy in furnishing compounds.

In table 5 the results for all ten drugs, together with the data on control animals, are summarized and listed in order of anticoliform activity, from high to low, on the basis of equal concentrations of drug in the test diets. Detailed results on each individual mouse are given elsewhere.[3] With each drug-diet, it is evident that, on the average, the pre-treatment and post-treatment coliform counts differed significantly; i.e., each drug produced a significant lowering of the coliform flora. It is also evident that each of the sulfonamides tested produced, on the average, a reduction in the coliform count which was significantly greater than the reduction which might have been expected in untreated mice. Thus, the anticoliform effect ranged from slight activity on the part of sulfanilamide (about a ten-fold reduction) to a high degree of activity on the part of sulfapyrazine (almost a million-fold reduction).

From the data in table 1, it was seen that all of the drug-diets were tested in groups of mice which were similarly composed with respect to pre-treatment coliform counts. Therefore, a qualitative comparison of drug activity may be made. Using the average reduction in count as the criterion of activity, a comparison on the basis of equal amounts of drug fed in the diet indicates that sulfapyrazine, sulfadiazine and sulfathiazole were all more active than sulfaguanidine, sulfanilylarsanilic acid, succinylsulfathiazole and sulfanilamide. Basing the comparison on drug intake, or actual dosage, does not change the relative activity of these drugs, since the intake of the first three drugs

was equivalent to, or less than, the intake of the last four drugs. Comparative activity, on the basis of the concentration of drugs in the feces, cannot be determined from the data at hand because of differences in absorption and solubility. Sulfanilamide, although least active on the basis of concentration in the diet, might well be relatively

TABLE 5

Sulfonamide activity against coliform bacteria in the intestines of mice

1 PER CENT DRUG-DIET	MEAN DRUG INTAKE	DRUG CONC. IN FILTERED STOOL SUSPENSIONS	DRUG* IN WATER CONTENT OF STOOL SAMPLE	LOGARITHMIC COLIFORM COUNT PER 9 CC. OF STOOL SUSPENSION			
				Before treatment	After treatment	Reduction	t†
	mgm./day	*mgm./100 cc.*	*gm./100 cc.*				
Sulfapyrazine	24.2	2.82	1.71	6.50 ±.30	0.70 ±.25	5.80±.25	14.9
Sulfadiazine	36.1	1.72	1.05	5.95 ±.30	0.65 ±.22	5.30±.29	18.3
Sulfathiazole	38.9	0.30	0.18	5.95 ±.34	1.20 ±.36	4.75±.45	10.6
N^1 Benzoylsulfanilamide	41.1	0.39	0.24	6.30 ±.31	1.70 ±.44	4.60±.39	11.8
Sulfapyridine	37.8	0.62	0.37	6.60 ±.41	2.70 ±.43	3.90±.61	6.4
N^1 Acetylsulfanilamide	45.2	0.44	0.27	6.95 ±.34	3.15 ±.54	3.80±.59	6.4
Sulfaguanidine	50.0	3.00	1.82	5.80 ±.34	2.40 ±.48	3.40±.40	8.5
Sulfanilylarsanilic acid	38.0	5.80	3.52	6.40 ±.39	3.50 ±.51	2.90±.55	5.3
N^4 Succinylsulfathiazole	45.8	2.61	1.58	6.35 ±.32	3.80 ±.52	2.55±.53	4.8
Sulfanilamide	39.3	0.27	0.16	6.65 ±.42	5.50 ±.33	1.15±.47	2.4
Control diet				6.18 ±.25	6.20 ±.27	−0.02±.32	0.6

Figures above are mean values, each based on 20 mice with the exception of values for the control diet, which are based on 40 mice. Logarithmic counts and reductions are given with their standard errors. In each case, the mice were fed on drug-diet for a period of 5 days.

* Calculated, on the assumption that 100 cc. of standard stool suspension contained 0.275 grams of wet stool with a stool water content of 60 per cent.

† A t value of 2.09 represents a probability of 5 per cent for occurrence by chance on the assumption that the "before" and "after" coliform counts were homogeneous.

more active, if comparisons could be based upon drug saturation of the intestinal contents.

If the number of mice which showed a reduction of at least 100,000-fold in coliform count is used as the criterion of drug-diet activity, the drugs may be listed, according to their relative effectiveness, as shown

in table 6. It is interesting to note that the relative activity of each drug, based on the 100,000-fold reduction response, is approximately the same as it was when based on the average amount of reduction in coliform count per mouse.

Each of a number of other drugs, including hexylresorcinol, carbarsone, N^1 (2-hydroxy) phenyl sulfanilamide, N^1 (3-hydroxy) phenyl sulfanilamide, and N^1 (4-hydroxy) phenyl sulfanilamide were tested for anticoliform activity in five mice. A significant reduction of the

TABLE 6

Sulfonamide activity against coliform bacteria in the intestines of mice

	NUMBER OF MICE TESTED	MICE SHOWING REDUCTION OF 100,000-FOLD, OR GREATER, IN COLIFORM COUNT	
		Number	Per cent
Sulfapyrazine	20	17	85
Sulfadiazine	20	15	75
Sulfathiazole	20	12	60
N^1 Benzoylsulfanilamide	20	12	60
Sulfapyridine	20	9	45
N^1 Acetylsulfanilamide	20	8	40
Sulfaguanidine	20	6	30
Sulfanilylarsanilic acid	20	5	25
N^4 Succinylsulfathiazole	20	5	25
Sulfanilamide	20	0	0
Control diet	40	0	0

The mice were placed on each of the above drug-diets for a period of 5 days.

pre-treatment coliform count was not obtained with any of these compounds.

In table 5, the values for drug concentration in the stool samples were calculated as free drug, with the exception of the value for succinylsulfathiazole, which was calculated as total drug (i.e., as both conjugated and free sulfathiazole). The average value for total succinylsulfathiazole in the stool samples was 1580 milligrams per cent, calculated on the basis of stool water content. Determinations of free sulfathiazole in the stool samples from the mice fed on the succinylsulfathiazole diet, gave an average value of 75 milligrams per cent. It is of interest to note that the anticoliform activity of the succinylsulfathiazole diet may have been due entirely to the amount of free sul-

fathiazole which was apparently split off, in the gut, from the conjugated form of the compound. This is shown in table 7, where the average values for the free sulfathiazole concentrations in the stool samples of mice on the sulfathiazole and on the succinylsulfathiazole diets are given together with the corresponding reductions in coliform

TABLE 7

Relation between the sulfathiazole concentration and the reduction of coliform count in stool samples from mice on sulfathiazole and on succinylsulfathiazole drug-diets

	SULFATHIAZOLE* IN WATER CONTENT OF STOOL SAMPLE	REDUCTION IN LOGARITHMIC COLIFORM COUNT PER 9 CC. OF STOOL SUSPENSION
	mgm./100 cc.	
Sulfathiazole	210	5.4
Sulfathiazole	145	4.1
Succinylsulfathiazole	100	3.3
Succinylsulfathiazole	50	1.8

Figures above are average values, each based on 10 mice.

* Calculated, on the assumption that 100 cc. of standard stool suspension contained 0.275 grams of wet stool with a stool water content of 60 per cent.

TABLE 8

Relation between drug intake and reduction of coliform count with sulfaguanidine drug-diet treatment

PER CENT DRUG-DIET	DRUG INTAKE	DRUG* IN WATER CONTENT OF STOOL SAMPLE	REDUCTION IN LOGARITHMIC COLIFORM COUNT PER 9 CC. OF STOOL SUSPENSION
	mgm./day	*gm./100 cc.*	
1.00	51.5	2.58	3.8
0.50	26.0	1.05	2.1
0.25	12.4	0.58	0.4
Control			−0.2

Figures above are mean values, each based on 10 mice.

* Calculated, on the assumption that 100 cc. of standard stool suspension contained 0.275 grams of wet stool with a stool water content of 60 per cent.

count. It is apparent from these data that the degree of response obtained with succinylsulfathiazole is what might have been expected on the basis of the amount of free sulfathiazole in the stool.

III. Relation between drug intake and reduction of coliform count. In order to investigate the possibility of obtaining a

quantitative comparison of the anticoliform activity of drugs in the intestines of mice, three groups, of 10 mice each, were placed on drug-diets containing 1.0, 0.5, and 0.25 per cent of sulfaguanidine, respectively. A fourth group, of 10 mice, was kept on control diet. Treatment was carried out for a period of 5 days. The drug-diet intake and drug concentration in the stool were calculated for each mouse. Response to drug treatment was measured in terms of the difference between logarithmic coliform counts on stool samples taken before and after treatment. The results of this experiment are given in table 8. These results indicate good correlation between drug intake (dosage) and reduction of coliform count (response). The response also appears to be correlated with the amount of drug present in the stool sample.

DISCUSSION

In the present investigation, a method has been developed for measuring coliform densities by means of a lactose broth dilution count, the same series of tubes being used for both quantitative dilution and for culture of coliform bacteria from standard filtered suspensions of stool samples. Thus, a considerable saving in labor and material is obtained and drugs can be tested in relatively large numbers of animals. Presumptive coliform counts, based on gas production from the fermentation of lactose at 44° C., are readily confirmed by subculture to Eosin-Methylene Blue agar. The *Aerobacter* group of organisms are usually inhibited during incubation in a water bath at this temperature. Expression of coliform counts, obtained in this way, as logarithmic units (to the base 10), i.e., in terms of growth resulting from successive ten-fold dilution and culture, involves a sacrifice of precision. However, in view of the fact that drugs such as sulfapyrazine and sulfadiazine may produce a reduction in coliform count of 100,000-fold, or greater, it would appear that greater precision than that obtained by a dilution count is not required in this type of work.

Expression of the densities of coliform bacteria in the feces of mice in terms of actual numbers results in data which covers an enormous range between extreme values. Under these conditions, the arithmetic mean can not be used to express the typical value; a more typical average, and one which is less affected by extreme items, is the geometric mean, or mean proportion. Therefore, in the present study,

drug activity has been expressed in terms of reductions of average logarithmic counts, i.e., as logarithmic reductions of the geometric mean values for actual numbers of bacteria.

Considerable fluctuation of coliform content apparently occurs, from day to day, in the intestines of individual mice (table 3). This indicates that the validity of the results obtained with any method of determining anticoliform activity in these animals, depends to a large extent upon the use of adequate numbers of animals.

It has been shown previously (10), that sulfonamide therapy, in systemic bacterial infections, is based upon the maintenance of an effective concentration of drug in the blood and tissues for an adequate period of time. It has been assumed that the antibacterial activity of drugs in the bowel similarly depends on the maintenance of an effective concentration of drug in the intestinal contents. Since it has been found (9) that mice will eat a drug-diet with sufficient regularity and frequency to insure the continuous presence of drug in the intestines, this method of treatment was adapted to the present study. As indicated above, the response to this treatment has been measured in terms of the average difference between pre-treatment and post-treatment logarithmic coliform counts.

It was found that, on the average, the coliform count of untreated mice did not show a significant change at the end of the experimental period of 5 days. Therefore, a drug was considered to be active if it produced, on the average, a reduction in logarithmic count significantly greater than zero.

A qualitative comparison of the activity of ten sulfonamide drugs is given in table 5. On the basis of concentration in the diet, and also on the basis of drug intake, it is evident that sulfapyrazine, sulfadiazine, sulfathiazole and N^1 benzoylsulfanilamide are all highly active against coliform bacteria in the intestines of mice. The other drugs tested may be listed, according to the degree of activity shown, from high to low, in the following order: sulfapyridine, N^1 acetylsulfanilamide, sulfaguanidine, sulfanilylarsanilic acid, succinylsulfathiazole and sulfanilamide. On the basis of drug content in the stool, it appears that sulfapyrazine, sulfadiazine, sulfathiazole and benzoylsulfanilamide are more active than sulfaguanidine, sulfanilylarsanilic acid and succinylsulfathiazole against coliform bacteria in the intestines of mice.

In addition to its bactericidal activity against coliform bacteria in the gut, the inter-related factors of toxicity, absorption, excretion, solubility, and stability of a drug condition any preliminary assessment of therapeutic usefulness. Given a drug which is relatively nontoxic, poorly absorbed from the intestine and quite soluble in water, an argument for or against its therapeutic value could be based upon a combination of two kinds of experimental evidence: 1) its comparative bactericidal activity *in vitro* against coliform bacteria and intestinal pathogens; 2) its bactericidal activity in the intestines against coliform bacteria. It is conceivable that, if a drug produced a marked bactericidal effect on the coliform content of the normal gut and also produced, *in vitro*, a greater bactericidal effect on intestinal pathogens than on coliform bacteria, such a drug might be effective against the same pathogens under actual conditions of infection. However, such evidence can, at best, be only indicative since the final assessment of chemotherapeutic value can come only from carefully controlled clinical trials.

The activity of these sulfonamides against *E. coli in vitro* has been determined by a method previously described (13). The relative *in vitro* activity of some of these drugs against *E. coli* has also been reported by Poth and his coworkers (7). A comparison of *in vitro* activity ratios, based on sulfanilamide, from both sources, is given below:

	ESCHERICHIA COLI IN VITRO ACTIVITY RATIOS	
	(*Poth et al.*)	(*White et al.*)
Sulfanilamide	1	1
Sulfanilylarsanilic acid		$\frac{1}{4}$
Succinylsulfathiazole	$\frac{1}{10}$	1
Sulfaguanidine	2	2
N^1 Acetylsulfanilamide		8
Sulfapyridine	20	16
N^1 Benzoylsulfanilamide		16
Sulfathiazole	50	64
Sulfadiazine	20	64
Sulfapyrazine		64

It is of interest to note that the relative *in vitro* activity of these compounds, as recorded above, is in qualitative agreement with their *in vivo* activity, to the extent that the most active compounds *in*

vitro were found to be similarly most active in mice, on the basis of drug intake. It should be emphasized, however, that relative *in vitro* activity can not be used as a reliable guide to therapeutic usefulness in the intestines, since the rates and degrees of absorption and excretion of different compounds may vary widely in the same species of animal. It should also be emphasized that the relative activity of a compound, as determined in mice, may not hold for another species of animal, since the rate and degree of absorption and excretion of the same compound may vary from species to species. A reliable experimental assessment of the therapeutic value of a drug can only be made from a combination of several different kinds of evidence.

Under the conditions of the experiments presented here, the activity of succinylsulfathiazole, both *in vitro* and *in vivo*, is apparently due to the amount of free sulfathiazole present in each case. It has been shown (14) that, in general, when the free aryl amino group of sulfanilamide and its active derivatives is blocked by conjugation, activity may be explained on the basis of conversion of a part of the conjugated compound into an active product which possesses the free aryl amino group. Under *in vitro* test conditions, conversion of only 0.1 per cent of a succinylsulfathiazole solution would be required to account for the relative activity ascribed to this compound. In the mouse, it has been found that, on the average, about 5 per cent of succinylsulfathiazole was converted to free sulfathiazole and here, again, it was possible to account for activity on the basis of free drug alone.

A comparison of the *in vivo* activity of N^1 benzoylsulfanilamide with that of sulfaguanidine indicates that the former drug is more active. This finding does not confirm the conclusion of Brownlee and Tonkin (4) who reported the drugs as equally active against coliform bacteria in the intestines of mice. This discrepancy may be explained by the fact that these investigators based their comparison on results obtained with a small number of animals.

From the data presented in table 8 it appears that the anticoliform activity of drugs may be compared quantitatively by determining for each drug the dosage required to give the same response.

CONCLUSIONS

The activity of sulfonamide drugs against the intestinal coliform flora of mice can be determined quantitatively by use of the drug-diet

method of treatment together with a lactose broth dilution method of measuring bacterial densities in stool samples.

On the basis of either drug concentration in the diet, or drug intake per mouse per day, the compounds tested in this investigation may be listed, according to the degree of activity shown, from high to low, in the following order: sulfapyrazine, sulfadiazine, sulfathiazole, N^1 benzoylsulfanilamide, sulfapyridine, N^1 acetylsulfanilamide, sulfaguanidine, sulfanilylarsanilic acid, succinylsulfathiazole and sulfanilamide.

Under the experimental conditions of this study, the anticoliform activity of succinylsulfathiazole was apparently due to its partial conversion to free sulfathiazole.

The comparative *in vivo* activity of the sulfonamides tested was found to be in qualitative agreement with their comparative *in vitro* activity against *E. coli*.

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MYOCARDIAL NECROSES IN RATS ON A POTASSIUM LOW DIET PREVENTED BY THIAMINE DEFICIENCY

RICHARD H. FOLLIS, JR.

From the Department of Pathology, The Johns Hopkins Medical School, Baltimore, Md.

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It is well established that when certain animals (rats (1), mice (2), and swine (3)) are placed on a diet deficient in potassium (K), necroses of the myocardium occur, as well as lesions in the renal tubular epithelium. Several recent studies have also indicated that similar cardiac changes may be produced in the hearts of thiamine deficient animals (pigeons (4), dogs (5), foxes (6) and swine (7)). The experiments to be reported here were begun with the idea that, if the double deficiency of both K and thiamine was produced, the myocardial changes might be even more extensive.

MATERIAL AND METHODS

The basal diet used has been described elsewhere by Orent-Keiles and McCollum (8). Its composition is as follows: Lactalbumin (Harris), 10.0; wheat gluten, 4.0; gelatin, 4.0; sweet butter fat, 8.0; K free salt mixture, 4.6, choline hydrochloride, 0.2 and sucrose (Dyno) to make 100 parts. Fifteen drops of cod liver oil are added to each kilogram of diet. Instead of the liver concentrate employed by Orent-Keiles and McCollum (8) the following weekly supplements of crystalline vitamins[1] were given orally twice a week: riboflavin, 0.28 mgm.; calcium pantothenate, 1.0 mgm.; pyridoxine, 0.2 mgm.; and nicotonic acid, 1.0 mgm. One milligram of alpha-tocopherol was administered once weekly to each animal. The basal diet was supplemented as follows for the various control groups: thiamine deficiency alone, basal diet +.85% KCl; K deficiency alone, basal diet +0.2 mgm. thiamine hydrochloride per week; normal control group, basal diet +.85% KCl and 0.2 mgm. thiamine hydrochloride per week.

A total of 46 albino rats of mixed sex, weighing about 35–50 grams was used. All were kept in screen bottom cages. Food and distilled water were administered *ad libitum* except in a group of 5 K deficient animals in which the food consumption was restricted so that they failed to gain over a 2 week period. The number of rats studied in each group was as follows: K-thiamine deficient group, 16 animals; thiamine deficient group, 7 animals; K deficient group, 17 animals; control group, 6 animals.

[1] Furnished through the courtesy of Dr. D. F. Robertson, Merck and Co.

Sections of the heart, voluntary muscle and kidneys were studied in all the animals. Sections of all the tissues were studied in several animals in each group.

EXPERIMENTAL RESULTS

K-thiamine deficient group: These animals grew poorly, averaging a gain of 3.5 grams the first week and 0.5 gram during the second week. At the end of this time the weight became stationary or began to fall off. The average length of life was 24 days. There was a good deal of variation, one animal dying after 13 days on the diet while 2 lived as long as 42 days (Table I). In general those animals weighing

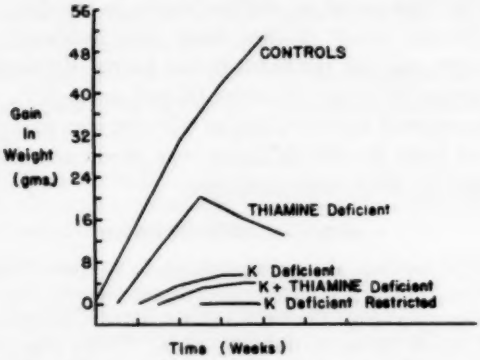

CHART I. GROWTH CURVES OF CONTROL GROUP AND VARIOUS DEFICIENT GROUPS

most at the beginning of the experiment lived longer than the rats weighing less at the outset. Paralysis was not noted in any animals.

Lesions were found microscopically in the heart of only one animal in this group and the lesions even in this animal which lived 29 days were minute. Three of the rats in this series were given 5 mgm. thiamine after 39 days on the doubly deficient diet. One died suddenly during the night and showed no cardiac changes. The other two exhibited many fresh lesions when autopsied 3 days later.

Changes characteristic of K deficiency were found in the kidneys of all the rats in this group.

Another interesting feature in these animals was the presence of necrotic voluntary muscle fibers in all but two. These consisted of

hyaline fibres surrounded by leucocytes. They appeared to be identical with the changes which have been described in vitamin E deficiency in young (9) and old rats (10). In only one animal (No. 13) were calcified fibers noted.

TABLE I

Duration of life, weight changes and pathological findings in rats on the K deficient and K-thiamine deficient diets

K-DEFICIENT GROUP							K-THIAMINE DEFICIENT GROUP						
Number	Days on diet	Initial weight	Final weight	Pathological changes			Number	Days on diet	Initial weight	Final weight	Pathological changes		
				Heart	Kidney	Voluntary muscle					Heart	Kidney	Voluntary muscle
		grams	*grams*						*grams*	*grams*			
11	17	28	30	+	+	0	10	13	48	41	0	+	+++
9	18	37	37	+	+	0	17	14	32	35	0	+	+
3	20	40	44	+	+	0	16	15	35	41	0	+	+
6	22	44	50	+	+	0	14	19	32	38	0	+	+
15	24	30	33	+	+	0	15	19	44	46	0	+	+
1	25	49	54	+	+	0	9	22	44	49	0	+	+
4	26	52	60	+	+	0	13	22	44	53	0	+	+†
5	26	41	46	+	+	0	18	23	34	50	0	+	+
8	28	38	48	+	+	0	6	25	43	43	0	+	+
10	28	53	62	+	+	0	2	28	52	43	0	+	+
13	28	28	37	+	+	0	5	29	45	52	+	+	++
16	28	31	40	+	+	0	7	31	51	48	0	+	+
2R	10	47	48	0	+	0	11	31	52	52	0	+	+
6R	11	40	40	+	+	0	4*	40	48	47	0	+	0
1R	12	45	45	+	+	0	1*	42	46	46	+	+	+
3R	13	39	36	+	+	0	8*	42	50	58	+	+	0
4R	13	38	37	+	+	0							

R = Restricted food intake.
* Given 5 mgm. thiamine on 39th day.
† Calc.

K-deficient group: This consisted of 2 sets of animals: one was fed *ad libitum*, while in the other food intake was restricted so that no gain in weight took place during the 10 to 13 day observation period. The weight gain of the rats fed *ad libitum* was only a little better than that of the K-thiamine deficient group. The average survival time of the 12 animals was 25 days, with variations from 17 to 28 days

(Table I). Microscopically, lesions were observed in the heart and kidneys of all the animals. These changes were extensive and identical with those we (1) have described elsewhere. The voluntary muscles showed no change.

In the animals on the restricted food intake lesions were noted as early as the 11th day. Changes were also seen in one animal sacrificed on the 12th day and in 2 rats killed on the 13th day. These changes appeared in spite of no gain in weight and indicated that inanition was not responsible in preventing the lesions from developing.

Thiamine deficient group: These animals initially grew much better than the two preceding groups. Observations were made on 7 animals receiving the deficient diet for periods ranging from 22 to 82 days. Those animals of this group living longest were kept alive by giving small amounts of thiamine. In none of the animals were myocardial or renal changes observed, nor did we encounter lesions in the voluntary musculature. None showed any paralysis or ataxia. They appeared weak and in some the heart rate could be determined by palpation since it was so slow. Terminally some of the animals developed a sort of rigidity with arched backs and tended to walk with the forelegs and hind legs extended. No detailed studies have been made of the nervous tissues of these rats.

Control group: These animals gained well as will be seen from the growth curves. No lesions were found in the heart, kidneys or voluntary muscles.

DISCUSSION

From these experimental results it is quite plain that we achieved the opposite effect to that which we had expected. Thiamine deficiency, instead of producing more extensive lesions in the myocardium of K deficient rats, prevented the appearance of any lesions at all, except in one instance.

The question at once arises as to what the mechanism for this protective effect may be. Before mentioning several purely speculative possibilities it should be pointed out that it is unlikely that inanition was a factor. It is true that the K-thiamine deficient animals did not grow as well as those on the K deficient diet alone. However, when animals were placed on the latter diet and their food intake was

restricted so that they failed to gain at all, lesions appeared in the myocardium on the 11th day of the deficiency. No animals in the group fed the K deficient diet without thiamine died before this time.

In rats bradycardia is a constant accompaniment of thiamine deficiency (11). The cause of this is not at all clear. Lu (12) has shown that there is a definite relationship between the blood pyruvic acid level and the heart rate. However, it has not been established that the high pyruvate concentration in the blood is the cause per se of the bradycardia. In 2 animals Lu (12) reported that the administration of thiamine brought about a return of the heart rate to normal in 4 hours. The possibility arises as to whether a decrease in rate might decrease the cardiac metabolism so that the needs for K would be reduced. We (13) have observed more extensive cardiac lesions in K deficient rats that had been subjected to severe voluntary exercise than in unexercised animals on the same diet. It might be assumed that the heart rate was increased in the exercised animals, though exact measurements would be difficult to make.

There are few biochemical studies which give any information regarding any direct relationship between thiamine and K. Ballif and Gherscovici (14) claimed that there is an increase in the K content of brain tissue of B_1 deficient pigeons but this could not be confirmed by Kinnersley (15). It is of interest, however, to mention the studies of Mann and Quastel (16) on the aerobic formation of acetylcholine by brain slices from polyneuritic pigeons. When thiamine was added to the medium (bicarbonate-Locke-pyruvate) no change took place. When relatively high concentrations of K ions (0.03 M) were added to the system there was a reduction in acetylcholine formation. This could be restored to normal by the addition of thiamine. This would seem to indicate that there is a relationship between the concentration of K and the action of thiamine on this particular phase of metabolism.

One hardly needs mention the relationship of thiamine to carbohydrate metabolism. The importance of K in this type of metabolism should also be pointed out. Ashford and Dixon (17) found that when more K was added to brain slices in an aerobic Ringer-bicarbonate environment glycolysis was increased.

Because of the influence of the adrenal cortical hormone on K and

carbohydrate metabolism it is of interest that Lockwood and Hartman (18) found that the addition of cortical hormone to a diet in which the source of the B vitamins was autoclaved yeast caused an increase in weight. Goodsell (19) has recently reported an increase above normal in the weight of the adrenal cortex in thiamine deficient dogs as well as an increase in the concentration of steroid compounds in the gland as measured by the bitterling test. Since it is well known that adrenal cortical hormone produces a fall in serum K, such observations might possibly indicate a change in the K content of the blood in thiamine deficiency though this question has not been studied. If so, however, this would fit in with the protective relationship shown in our experiments, since in K deficiency the K content of the blood is lowered.

The importance of inorganic ions in enzyme reactions has been known for a long time. It might be mentioned that Orent-Keiles and McCollum (8) found a decrease in the Mg and K content of cardiac tissue in animals receiving a K low diet. Mg is important for the activity of thiamine pyrophosphate. It is thus likely that an imbalance of one ion causes an imbalance of others which may in turn affect enzyme systems.

Thiamine deficiency failed to protect the kidney from the effects of K deficiency. Again the cause for these changes is not clear.

The failure to find lesions in the hearts of the thiamine deficient rats is not in keeping with what has been reported in other animals. However, physiological changes do occur as evidenced by alterations in the electrocardiogram (20) and a decrease in oxygen consumption of the myocardium (21).

The muscular dystrophy which was observed in most of the animals receiving the K-thiamine deficient diet is similar to that which has been described in vitamin E deficiency in rats (9) (10) and other species. It seems unlikely that a deficiency of this vitamin was present since it was administered in adequate amounts and lesions have only been observed in young rats born of deficient mothers, or in old animals on a vitamin E free diet. We have never observed lesions in the voluntary musculature in K deficient rats, even when severe voluntary exercise was instituted (13) and no changes were observed in the animals deficient in thiamine alone.

SUMMARY

The production of thiamine deficiency together with potassium deficiency in rats exerted a protective effect in these animals in that no myocardial necroses such as are seen in potassium deficiency developed. Possible explanations for this "protective" effect are considered.

Unlike observations in certain other species no cardiac changes were observed in rats on a thiamine deficient diet.

Changes in the kidneys characteristic of potassium deficiency did develop in all animals whether potassium alone or potassium together with thiamine was lacking.

In all but 2 of the 16 potassium-thiamine deficient animals necroses of the voluntary muscle fibers were observed.

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NOTE ON ABSENCE OF SYPHILITIC REAGIN AND ANTIGENIC SUBSTANCE IN URINE

VIRGIL SCOTT, M.D.*[1]

From the Syphilis Division of the Department of Medicine, The Johns Hopkins University

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During the early years following the discovery of complement fixation by Bordet and Gengou and its application to the diagnosis of syphilis by Wassermann, several investigators reported the presence of a complement-binding substance in the urines of patients with syphilis. Blumenthal and Wile (1) found that the urine of 41 out of 50 patients in various stages of syphilis gave positive complement fixation tests. A high percentage of positive reactions was also reported by Simon (2), who tested the urines of 156 patients with syphilis. The percentage of positive complement fixation tests ranged from 16 per cent in primary syphilis to 60 per cent in congenital syphilis. Bauer and Hirsch (3), however, found that only the urines of syphilitic patients which contained large amounts of protein (? syphilitic nephrosis) gave positive Wassermann reactions. The latter stated that the reacting substance was associated with the globulin fraction. In addition they pointed out that highly acid urine was frequently anti-complementary. This anti-complementary activity could be prevented by neutralization of the urine prior to the performance of the tests. Klauder and Kolmer[4] after studying the urines of 60 patients with syphilis concurred that urine is highly anti-complementary. Because of this fact false positive tests were not infrequent in their experience. Only two of the sixty patients tested were found to give positive reactions under technical conditions which they considered acceptable.

The present study was undertaken primarily in the hope that it might be possible to demonstrate the presence of an antigenic substance in the urines of patients with early syphilis by means of complement fixation. Because of the discordant views recorded above simultaneous testing of the urines for reagin was undertaken. The urines of 23 patients with syphilis and of 8 non-syphilitic controls were tested.

[1] E. R. Squibb Fellow in Medicine.

METHOD

An 18 hour ice-box complement fixation test was employed.

REAGENTS

Urine. Freshly voided urine was used. In the few instances in which this was not practicable the urines were stored for not longer than two hours at +5°C. No preservative was added.

To obviate the anti-complementary effect of acidity or alkalinity the pH of the urine was adjusted to 6.8 or 7.0 with N/10 NaOH or N/10 HCl using bromthymol blue as an indicator.

Complement. Pooled guinea pig serum was preserved by salting (.008 gm. sodium chloride per 1.0 ml. serum) and freezing at −25°C. Immediately prior to the test the frozen serum was melted in a water bath at 37°C. and diluted one to eight with tap water.

FIGURE 1

	TUBE NO.									
	1	2	3	4	5	6	7	8	9	10
Urine	0.2	0.1	0.05	0.2	0.1	0.05	0.4	0.2	0.1	0.05
Normal saline		0.1	0.15		0.1	0.15		0.2	0.3	0.35
Complement	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Antigen	0.2	0.2	0.2							
Positive serum				0.2	0.2	0.2				

Antigen. Beef heart antigen was sensitized by the addition of 0.2 per cent cholesterol. The optimal dilution for this antigen was found to be one part of antigen to 160 parts of normal saline.

Positive serum. The titer of a known positive serum was determined by the Eagle macroflocculation method. Only high titer (64–128 units) serum was used. The serum was preserved by freezing in small test tubes at −25°C. When needed the desired quantity was melted in a 37°C. water bath. In order to reduce the anti-complementary effect to a minimum the serum was diluted with normal saline so that each 0.2 ml. contained approximately 12 units of reagin. This diluted serum was used in the test and for the positive serum control.

TEST

The test was set up in the manner indicated in figure 1.

CONTROLS

The controls for anti-complementary activity of the urine are included in figure 1, tubes 7–10.

In addition the following controls were run with each test:

1. Serum control for anti-complementary activity in two dilutions—0.4 ml. and 0.2 ml.
2. Known positive (see above) and known negative serum controls.
3. Complement controls using four dilutions (0.2 ml. to 0.05 ml.) of complement.
4. Complement-antigen controls (Hemolytic Indicator) using dilutions of complement as in (3) with the addition of 0.2 ml. of antigen to each tube.

The test proper and the controls were incubated in the ice box at +5°C. for 18 hours. The tubes were then placed in a water bath at 37°C. for 30 minutes. 0.4 ml. of sheep red blood cells sensitized with 2 units of amboceptor was added to each. Further incubation in the water bath at 37°C. (10–30 minutes) was governed by the hemolytic indicator.

RESULTS

The urines of twenty-three patients with syphilis and of eight non-syphilitic controls have been tested by this method. The stage of

TABLE 1

Diagnosis	Albumin (Esbach Method)	Units of reagin in blood serum (Eagle macroflocculation method)
	gm./liter	
Syphilis, secondary, syphilitic nephrosis	2.0	48
Syphilis, latent, late. Laennec's cirrhosis	0.25	32
Syphilis, late, cardiovascular aortic regurgitation, congestive failure	1.5	24
Syphilis, latent, late. Malignant nephrosclerosis	1.0	32
Syphilis, primary	0.25	32

syphilis was distributed as follows: primary, seronegative, 2; primary, seropositive, 2; secondary (all seropositive), 14; latent, late, 2; late, cardio-vascular, 1; late, gumma of eyelid, 1. The reagin content of 16 of the seropositive sera was determined by the Eagle macroflocculation technique. As most of the patients had early syphilis a high titer was the rule. The average in this group was 54+ units of reagin with extremes of 2 and 192 units.

The urines of all patients were routinely tested for the presence of albumin (heat and acetic acid method). Only five patients were found to have albuminuria, and in no instance was a large amount of albumin present. Partition of the urinary protein into albumin and globulin fractions was not done. A summary of these five patients is presented in table 1.

In none of the urines tested was it possible to demonstrate either reagin or an antigenic substance by this method. Urine was found to be frequently anti-complementary despite neutralization. This anti-complementary activity seemed to be directly proportional to the amount of urine present. Incomplete hemolysis occurring in the "test" tubes could be attributed only to either the anti-complementary activity of urine alone or to a summation of the anti-complementary action of urine plus antigen or positive serum.

Despite the absence of reagin from the five urines containing protein, it is entirely conceivable that an occasional such patient may excrete demonstrable amounts of reagin. For this to occur two factors would seem to be necessary—first, a relatively high percentage of globulin in the excreted protein, which presupposes severe damage to the glomerular filter; and second, a high reagin content in the blood serum. Neither would seem to have existed to a sufficient degree in this small series.

SUMMARY AND CONCLUSIONS

Complement fixation tests have been performed on the urines of 23 patients with syphilis and of 8 non-syphilitic controls. The presence of neither reagin nor an antigenic substance was detected by this method. Urine is frequently anti-complementary.

The author wishes to express gratitude to Dr. Alan M. Chesney, under whose direction this study was undertaken, and to Dr. Harry Eagle for invaluable technical advice.

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Message from 18th General Hospital concerning the death of

GENERAL JOHN M. T. FINNEY

Twenty-five years ago the members of old Base Hospital No. 18, American Expeditionary Forces, France, were looking to John M. T. Finney for the type of leadership that only he could give, and which became so familiar to every medical man in America, especially to those serving with our armed forces overseas. We can no longer look to Dr. Finney personally for the kind of friendly counsel, advice and leadership that was so characteristic of him, but the inspiration of his life, both as a surgeon and as a man, will linger as long as the name Johns Hopkins occupies a place in the medical firmament.

The members of the 18th General Hospital, now serving overseas with our armed forces in the far Pacific in another World War, cannot help feeling but that Dr. Finney's mantle has in a degree fallen on our shoulders. Our most ambitious hope is that we may in a measure live up to the high standard set by him for the old unit. God grant that this hope may be realized!

AMOS R. KOONTZ, Lt. Col., M.C.
Commanding—18th General Hospital

BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal.)

The Dynamic State of Body Constituents. By RUDOLF SCHOENHEIMER. *Harvard University Monographs in Medicine and Public Health.* 78 pp. $1.75. *Harvard University Press,* Cambridge, Mass., 1942

The three lectures contained in this volume admirably summarize the numerous publications of the late Dr. Schoenheimer and his associates. The reviewer finds the book a laudable commentary on the foresight of Schoenheimer in grasping the usefulness of isotopic compounds as tracer substances in biological reactions and the skill of his group in synthesizing the isotopic compounds and in devising methods and apparatus for their subsequent isolation and analysis in body tissues. For an adequate appreciation of these details the reader is urged to examine the original papers in his field of particular interest.

The first lecture covers the investigations into the metabolism of the steroids and fatty acids. It was while working in this field that the notion of the existence of a state of rapid flux of body metabolites first impressed itself on the author. In the second lecture is found a concise and clear exposition of the numerous experiments conducted in the field of intermediary protein and amino acid metabolism of animals with the aid of isotopes. Strong arguments and evidence against the concept of independent exogenous and endogenous types of metabolism are made. The new concept of the mobility of all amino nitrogen except that of lysine receives ample experimental justification. The idea of the dynamic state of the body constituents is given further factual proof in this lecture. The final lecture on the formation of excretory products describes the finest work of this group of investigators, namely,—the isotopic confirmation of the Krebs-Hensleit cycle for urea formation and the biological synthesis of creatine.

These collaborate activities represent the most prodigious advance in the understanding of the utilization and formation of the animal body metabolites within the last decade. Consequently, teachers of biochemistry will discover in this book valuable advance source material for their lectures. Investigators should find it a useful and time saving survey of the experiments done by these workers at Columbia. Both groups may well be thankful for the efforts of Dr. H. T. Clarke who made the posthumous publication of these lectures possible.

A. A. A.

Neural Mechanisms in Poliomyelitis. By HOWARD A. HOWE AND DAVID BODIAN. 234 pp. Illus. $3.50. The Commonwealth Fund, New York, 1942.

The authors record in monograph form a series of investigations on experimental poliomyelitis. The work deals primarily with neuro-virus relations and was

planned by men fundamentally trained in neuroanatomy and who have knowledge about virus diseases of the nervous system.

The publication contains eleven chapters which present experimental methods, results and interpretations on the neurotropism of poliomyelitis virus; mechanism and rate of spread in peripheral nerve; the behavior of virus in peripheral nerves and in non-nervous tissues; the genesis of cerebral lesions; the neurons in the dissemination of virus; the portal of entry; the pathology of early arrested and non-paralytic poliomyelitis; refractory states in nerve cells and certain problems in immunity to poliomyelitis.

Pertinent questions in regard to virus-host reactions and relationships are asked which are applicable to many problems on infectious disease.

The nerve section and immersion methods of virus exposure should be of interest especially to those interested in neurotropic agents and may be of considerable use in immunity experiments.

Good evidence is presented that the axis-cylinder transmits poliomyelitis virus to the nerve cell body and that the number and size of the fibers as well as immersion time of exposure are significant when the intraneural route is used. Apparently both motor and sensory fibers can transmit the virus equally well, while sympathetic fibers are not especially susceptible to the virus.

It is shown by the method of retrograde degeneration in the thalamus that poliomyelitis virus is "neuronotropic" in its behavior since mesodermalglial inflammation seemed to be of secondary nature.

A possibly significant observation in regard to portal of entry has developed from the work on the pattern of virus dissemination, in that ascending and descending infections gave somewhat different histo-pathological pictures.

The rhesus monkey, in so far as portal of entry studies go, loses ground as an experimental animal since it is susceptible only to intranasal exposure, while the chimpanzee seems to be comparable to man in that this species is susceptible to the virus by oral, gastro-intestinal and intranasal inoculation. Pathological changes, however, in the monkey as well as in the chimpanzee are nearly comparable after the virus enters the central nervous system and may or may not cause paralytic attacks.

The authors suggest that the problem of resistance to poliomyelitis in man is not so much concerned with the reaction of the nervous system as it is one of preventing the virus from entering the nervous tissues. However, it is conceivable that any change produced in the cells of nervous tissue which would permanently alter their metabolism from the normal would result in a refractory state. This view is particularly important in relation to many of the filterable viruses since it is generally agreed that they fail to multiply to any extent in abnormal or sick cells, particularly those viruses in which the highest form of parasitism is necessary for their propagation.

R. E. G.

Textbook of Clinical Parasitology. By DAVID L. BELDING. 888 pp. Illus. $8.50. D. Appleton-Century Company, New York, 1942.

The clinical aspects of the various parasitic infections are more effectively analyzed in relation to conflicting syndromes than is usually attempted in parasitological texts. Nevertheless, this newest addition to the available texts in this field exceeds the strict interpretation of its title by a wide margin. The biology of the organisms considered has been adequately covered despite the statement in the preface that the "zoology of the parasite has been subordinated." In those cases in which a clear cut clinical syndrome is absent a full discussion is, nevertheless, given of the biology of the parasite and the resulting infection.

The text is organized in seven sections. The first, entitled "General Parasitology," includes, besides a general introduction, chapters on Pathology, Immunology, Transmission, Diagnosis, and Treatment and Prevention. This is followed by the usual sections on Protozoa, Roundworms, Tapeworms, Flukes, and Arthropods; each of these sections is introduced by a chapter on the morphological taxonomic, biological, and medical aspects of the group considered. Such general discussions are helpful but they have been carried to the extent of being repetitious and even redundant, adding to the length of an already long text. The final sections cover effectively the laboratory aids to the identification of the parasites and the diagnosis of the infections.

The author has been singularly successful for the most part in keeping the student point of view in mind. The summary tabulations which are an outstanding characteristic of the book permit the reader to compare at a glance life histories, diagnostic characteristics, clinical manifestations, geographical distribution, and many other phases of the subject. The effort required has been more than justified, in the opinion of the reviewer. Another outstanding characteristic of the book is the collection of extra-ordinarily numerous and effective illustrations; these alone make the book extremely valuable. The subject matter has been brought up to date and the text is replete in references to current literature. For the most part the current literature has been well handled but in some cases abstracting rather than interpreting the literature leaves the reader with a confusion of words rather than the core of an idea.

Apparently some errors are unavoidable in a new edition, but comparatively few were noted. Under this heading, however, the reviewer was surprised that the de Rivas hot water therapy in any of its modifications is seriously considered for Giardiasis (page 132) or for any enteric infection.

On the whole both the author and publisher are to be congratulated on producing so excellent a text. It should be well received not only by teachers and students in medical schools but by the more specialized student of parasitology as well.

G. F. O.

Manual of Standard Practice of Plastic and Maxillofacial Surgery. Prepared and Edited by the Subcommittee on Plastic and Maxillofacial Surgery of the Com-

mittee on Surgery of the Division of Medical Sciences of the National Research Council, and Representatives of the Medical Department, U. S. Army. 432 pp. Illus. $5.00. W. B. Saunders Company, Philadelphia & London, 1942.

As stated in the introduction, "this volume is one of a series developed under the auspices of the Division of Medical Sciences of the National Research Council to furnish the medical departments of the United States Army and Navy with compact presentations of necessary information in the field of military surgery." This purpose is attained admirably.

The book is divided into four sections; reconstructive surgery of the head and neck, maxillary surgery, maxillofacial prosthesis, and anesthetic technics. In spite of multiple authorship the style is uniformly succinct and clear. The illustrations are excellent.

The authors have aimed to provide a standard of practice in plastic surgery which will meet the demands of most of the conditions which are encountered in military and in civil practice. They have sought to avoid perpetuating many past mistakes and to recognize both the good and the bad in literature and practice. They are to be congratulated on the result of their efforts. If the remainder of this series continues the same standard, a distinct contribution to surgical literature will be made.

E. M. H.

Diseases of Metabolism. Edited by GARFIELD G. DUNCAN. 985 pp. Illus. $12.00. W. B. Saunders Company, Philadelphia and London, 1942.

This book consists of a series of monographs by distinguished authors in the field of metabolic disorders. For the most part these articles are excellently prepared and careful attention is paid to the primary aim of the work—namely, to bring to the clinician the most recent laboratory research data in metabolic diseases.

In any subject in which there is so much current activity and advancement, there are bound to be controversial points if the review is brought up to date. The authors have, however, avoided particularly controversial subjects and, where this could not be done, have presented both sides with fairness.

We do not know of any current book where an equivalent amount of authoritative data on metabolic subjects may be so readily found. This work should prove of great reference value to practitioners of medicine, as well as to medical students—despite the fact that because it is so up to date, it will, we hope, need early revision.

J. E. H.

Rabies. By LESLIE T. WEBSTER. 168 pp. Illus. $1.75. The Macmillan Company, New York, 1942.

This small volume is a very concise and valuable review of much of the present knowledge concerning rabies. It deals chiefly with the methods for the diagnosis

of the disease in animals and discusses in a very clear manner its epidemiology. A summary of the experimental evidence for the value of vaccination prior to exposure is included, as well as a discussion of the value of vaccines following exposure to a rabid animal. It is pointed out by Webster, as has been previously done by McKendrick, that paralytic accidents are three or four times as common following the use of vaccines containing "living" virus as they are after vaccines composed of "killed" virus. For the practicing physician and Public Health authorities the most important question concerning rabies is when to advise anti-rabic vaccination and when to omit such treatment following exposure to an animal suspected of being rabid. In one of his chapters Dr. Webster gives a very thorough discussion of the many factors which require consideration in arriving at a solution to this problem.

H. L. H.

The Conquest of Bacteria. By F. SHERWOOD TAYLOR. 178 pp. Diagrams. $2.00. *Philosophical Library and Alliance Book Corporation, New York, 1942.*

A popular book on a scientific subject should always be accurate insofar as details are concerned. Otherwise, its readers, many of whom are bound to be uncritical, will be misled. This book contains some remarkable statements and inferences, as for example, that typhoid fever can be cured by medicine; that antibodies are limited to agglutinins and bacteriolysins; and that the use of sulfarsphenamine has supplanted arsphenamine. The author's treatment of the subject of the development of sulfonamide therapy is inaccurate in certain respects, and is not at all up to date. An example of this is his statement that sulfapyridine (M. & B. 693) seems to be one of the safest of the sulfonamide group of drugs, a remark which obviously was not true even at the time this book was written.

It seems to the reviewer that this book is interesting from the point of view of how careful one should be not to establish and perpetrate inaccuracies when writing a popular book on science.

P. H. L.

Doctor Bard of Hyde Park. By JOHN BRETT LANGSTAFF. 365 pp. Illus. $3.75 E. P. Dutton & Co., New York, 1942.

With this book the author, an Episcopalian clergyman, makes his first entrance into medical history. He writes of Dr. Samuel Bard (1742–1821), one of the outstanding physicians of New York City during the American Revolution and afterwards, who deserves to be remembered to-day not only for his skill and common sense in practicing medicine but for his sagacity and vision in sponsoring the establishment of medical and educational institutions in the colonial city.

While studying medicine in Edinburgh, Bard met John Morgan, another young American, who was planning to return to his native Philadelphia and to found a medical school, the first in the colonies. Bard immediately was seized with the desire to do the same in New York, and as a result the medical school of King's

College, later Columbia, was opened in 1767, two years after the Philadelphia school, with Bard among the six faculty members. Largely through his efforts, the New York Hospital, the College of Physicians and Surgeons, and the Elgin Botanic Garden (now the site of Rockefeller Center and still owned by Columbia University) were established.

Of great interest are Bard's connections with his contemporaries. He treated George Washington during a serious illness which befell him while residing in New York soon after he became President; he was a friend of Alexander Hamilton and Aaron Burr; he was associated with David Hosack, another prominent physician of the time; he had as his disciple Samuel Latham Mitchill, an early American scientist. He shunned politics and maintained a strict neutrality throughout the Revolution.

The later years of his life were spent on his estate at Hyde Park (familiar today as the home of President Roosevelt) which had been developed by his father. Always much interested in gardening and husbandry, Bard was a member of several agricultural societies and wrote a book on sheep-raising. The biographer has also included a great deal of material on his religious activities and on contemporary church history.

An exhaustive research of six years' duration through a great quantity of unpublished manuscript material in widely scattered places has enabled Mr. Langstaff to present a very detailed account of Bard's antecedents, his life and interests. At times one wishes that the writer had employed footnotes for some of the less pertinent facts. Innumerable quotations from contemporary letters and documents appear throughout the book. However useful these are in rendering a picture of the times and the idioms employed, the general effect is to reduce the fluidity of the narrative. But these are matters of style and do not destroy the value of the account.

One serious criticism, however, is that the book contains only a biographical index. If one is interested in contemporary events or in the history of newly founded societies, the facts are difficult to find.

Of great aid to the medical historian and those interested in early New York history is the very careful bibliography which the author has included. The well reproduced illustrations include several which have hitherto been unpublished.

G. M.

FACTORS INFLUENCING THE LEVEL OF VITAMIN A IN THE BLOOD OF RATS

HUGH W. JOSEPHS

From the Department of Pediatrics, Johns Hopkins University School of Medicine and the Harriet Lane Home, Johns Hopkins Hospital, Baltimore

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There has been an increasing tendency to consider the level of vitamin A in serum or plasma as the best guide to the diagnosis of vitamin A that we have at present. There is of course much evidence that it can be so used, but in spite of all the work that has been done, we still know too little of the factors that determine the blood level, and we also know little of the significance of the level in relation to deficiency. It has been determined that in humans a diet low in vitamin A over a long period of time will eventually cause a reduction of the blood level to a point below that which is considered normal (1, 2, personal observation). It has also been found that the blood level is likely to be lower in the poorer sections of the population than among the well-to-do (3, 4, 5, 6, 7). But when we are faced with a low or somewhat low value, we find ourselves unable to state its significance with any degree of certainty. Little by little as we learn the conditions under which low values occur, we may fill the gaps in our knowledge and we may be able eventually to say in one case that this is deficiency because stores are lacking; in another that deficiency exists because distribution is defective; in a third that the low value is of no significance because the needs are low; or again that it is of no significance because it is merely a temporary reaction brought about by certain conditions to which the organism will soon adjust itself.

From the point of view of the clinic the studies should be made under conditions that affect human beings, but there are certain problems for the solution of which we must turn to animals. Among these are the relation of blood level to storage and the relation of blood level to the development of deficiency. It is especially with these problems that this paper is concerned.

GENERAL PLAN

Rats were used throughout this work. It was planned to study 1) the effect of vitamin A ingestion on the level of vitamin A in the serum, 2) the effect of the concentration of vitamin A in the liver on the serum levels, 3) relation of the depletion of vitamin A in liver and serum to the development of deficiency. The rats were weaned at 22 days and were placed immediately on the basic vitamin A deficient diet. The rats that were given vitamin A supplement received it in the form of Abbott's or Mead's halibut liver oil, undiluted in the case of the large doses, diluted with corn oil in the case of the smaller doses to an extent such that the desired dose was contained in about 4 drops of the diluted oil (about .1 cc.). In every case the oil was administered individually by dropping measured amounts from a tuberculin syringe into the mouths of the rats.

The rats to be sacrificed were first anesthetized with ether. As soon as the rat was unconscious, the neck was cut and the blood collected in a beaker. The collected blood was allowed to clot, and the vitamin A and total lipid determined in the serum by methods which have been reported in detail in a previous communication (8). The liver was removed, weighed and a sample for vitamin A was taken. The sample was weighed, then ground up with sand and a small amount of 50 per cent alcohol in a small mortar. The alcoholic suspension was transferred to a Wassermann tube with small additional amounts of 50 per cent alcohol, the total amount of suspension amounting to about 4 cc. To this 1 cc. 95 per cent alcohol was added, then 2 cc. petroleum ether and the vitamin A was determined in the petroleum ether layer as in the case of serum.

The unit used in this work was obtained from a commercial sample of halibut liver oil containing not less than 50,000 international units of vitamin A per gram. It was assumed that 1 cc. contained 50,000 units and the unit used in this work was based on the galvanometer deflection produced by 50,000 of a cubic centimeter of this oil. It has been reported by a number of workers that the blue color of the Carr-Price reaction obeys Beer's Law only approximately and only over a limited range (9). This fact I can confirm from personal observation. For this reason values are obtained from a calibration curve which relates galvanometer deflection to the number of units present in the micro cell containing the fatty residue dissolved in 0.1 cc. chloroform and 1 cc. of antimony trichloride reagent. The unit used here must of course be considered arbitrary, since the exact amount of vitamin A in the sample used for calibration is not precisely known, but, judging by the results of others (10), this unit is close enough to the international unit to be considered equivalent for practical purposes.

DIET

The basic vitamin A free diet was as follows:

Casein (acid and alcohol washed, dried in the air and powdered)	180 gm.
Corn starch	650 gm.
Brewer's yeast	80 gm.

Salt mixture	50 gm.
Corn oil	50 gm.
Viosterol	2 cc.

The salt mixture was as follows:

Calcium carbonate	250 gm.
Potassium chloride	165 gm.
Sodium chloride	80 gm.
Sodium bicarbonate	115 gm.
Magnesium oxide	32 gm.
Ferric citrate	80 gm.
Monobasic potassium phosphate	280 gm.

RESULTS

1. Effect of vitamin A intake on vitamin A of blood

In this part of the work the rats were maintained after weaning on the basal deficient diet. In addition they were given vitamin A in different amounts by syringe as previously described. For the presentation of results they are divided into three groups: 1, maintenance levels, about 2–5 units daily; 2, moderate amounts, 20–100 units daily; 3, large amounts, 1,000 units or more daily.

As may be seen in chart 1, the levels in the serum of rats on "maintenance" and moderate doses were essentially the same:—between 0.5 and 1.0 units per cc. On the larger doses the serum level was appreciably higher. Since all the animals were killed 24 hours after the last dose of vitamin A it is possible that the higher serum levels in the rats on large vitamin A intake were due to the recent ingestion of the vitamin; i.e., that the vitamin ingested the previous day had not yet been removed from the blood stream. To what extent this was the case may be seen in chart 2, the figures for which were obtained from animals in which the high vitamin intake had been discontinued.

It is evident that after 2 or 3 days the vitamin A had fallen to the level characteristic of the lower range of intake. We may conclude therefore 1) that the vitamin A tends to be established under the conditions of these experiments at a level of .5 to 1.0 units per cc. of serum, and 2) that we may force the level to a higher point by an excessive administration which taxes the ability of the tissues to remove the vitamin from the blood, but that the level falls again relatively rapidly when the excessive administration is discontinued.

These observations are in essential accord with those of McCoord

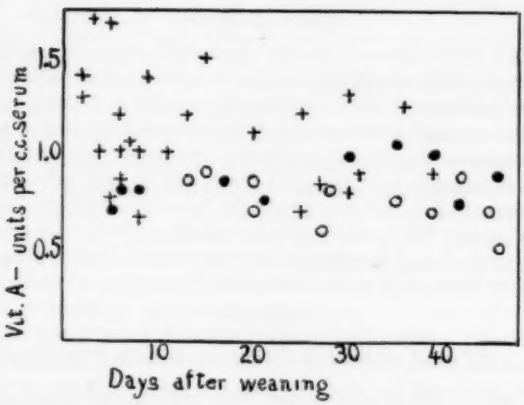

CHART 1. VITAMIN A OF SERUM IN RATS GIVEN DIFFERENT AMOUNTS OF VITAMIN A

Note: The hollow circles refer to the rats on "small" doses of vitamin A; the solid circles refer to rats on moderate doses; the crosses refer to rats on excessive doses (see text).

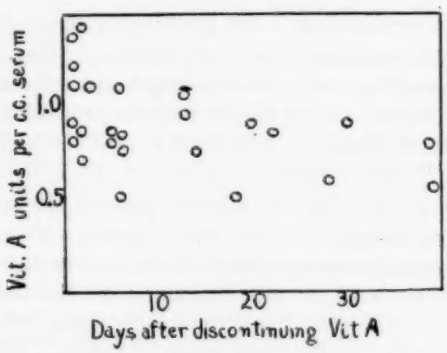

CHART 2. VITAMIN A OF SERUM OF RATS ON EXCESSIVE INTAKE AFTER DISCONTINUANCE OF ADMINISTRATION

Note: Since in this chart it is shown that when excessive intake of vitamin A is discontinued the serum level falls to "normal" values within 4 to 5 days, it has been inferred that the above-normal values of chart 3 were dependent primarily on the administration of excessive vitamin A.

and Luce-Clausen (11) and those of Brenner, Brookes and Roberts (16). Lewis, Bodansky, Falk and McGuire (12), however, found not only that the serum level was appreciably higher on moderate doses than on maintenance doses, but that the still higher values on large doses were maintained over a considerable time when the high dosage was discontinued. It is hard to explain the difference in results obtained after discontinuance of the high vitamin intake, unless it is due to duration of the high intake, which in my rats was only 2–3 weeks, whereas in those of Lewis and his co-workers it was 6 weeks. The few weeks difference in age of the rats was not enough to matter. With regard to the difference between minimal and moderate doses, it is possible that with more figures a difference in vitamin A levels in the serum might be established.

2. Relation of blood level to stores

The problem here is to determine to what extent the blood level is determined by the concentration of vitamin A in the tissues. For this purpose the liver is the only organ that need be considered (11, 13, 14). At first sight it would seem reasonable to suppose that the dependence of blood values on the habitual level of intake is due to the fact that the concentration in stores is also dependent on the amount of ingested vitamin A. Most workers, however, have found that there is little if any relation between the blood level of vitamin A and its concentration in the liver, as long as there is an appreciable amount of the vitamin present (11, 15, 16).[1]

This section of the work includes all of the rats studied on which vitamin A was determined both in the serum and liver. The intake of vitamin A varied from none at all up to the relatively large doses of 2000–3000 units per day. In the case of the large doses it seemed

[1] Lewis, Bodansky, Falk and McGuire (12) came to the conclusion that storage level has an influence on the blood level. This conclusion is based on the fact that the high blood level which resulted from ingestion of large doses of vitamin A was maintained over a long period after discontinuing the excessive intake. Horton, Murril and Curtis (21) are credited with showing a relation between storage and serum levels (16). They however made no such claim and what they actually showed was that in rats on deficient diets the blood and liver levels of vitamin A declined in a parallel manner. This is exactly what has been shown in the present work.

desirable to separate the figures for the blood levels that might have been influenced by recent ingestion from those in which no such influence would have been present. Accordingly in the case of high doses, the figures from rats in which the vitamin A had been discontinued for at least 2 days were included with those from rats receiving smaller doses, as representing data that could be used to show relationship between storage and blood level. The effect of recent ingestion of large doses is shown by the use of a different symbol, to represent figures from rats that had received the large doses of vitamin A to within two days of the determination.

As may be seen from chart 3, there is a wide range of values over which the concentration in the liver had no influence on the blood level apart from the cases in which recent ingestion was a possible factor. The rats in this group included some in which the storage was as high as 1200 units per gm. of liver; which is as high as Lewis and his co-workers obtained in their rats. It is not possible to be certain about the effect of still higher storage levels, because such figures were obtained only from animals which had been receiving excessive doses of vitamin A up to within two days of the determination. However judging by the distriubtion of the crosses in the chart it is evident that storage level had a marked influence on the blood level in these animals.

The most likely explanation for this appears to be that when the concentration in the tissues reaches a high level they become "saturated" to the extent that they are unable to take up more vitamin A as fast as it comes so that the freshly absorbed vitamin piles up to some extent in the serum.[2] However that this "saturation" is only relative is shown by the fact that the vitamin A of the tissues can be pushed to much higher concentrations if the excessive vitamin A administration is continued for a longer time. McCoord and Luce-Clausen (11) were able to push the vitamin A concentration in the liver to extremely high values without being able to show that such high values had any influence on the serum levels.

[2] Davies and Moore (14) considered that hypervitaminosis was dependent not on excessive amounts of vitamin A in the body but due to a rate of ingestion greater than could be taken care of by storage. Lasch and Roller (17) showed that when the cells of the reticulo-endothelial system were "blockaded" by various means, the percentage of administered vitamin A found to be stored was considerably reduced.

Another possible explanation exists, namely that the effect of excessive storage on serum level is not dependent on recent ingestion, and that the reason that the high serum values occurred only in the case of recent ingestion is that the storage concentration drops with

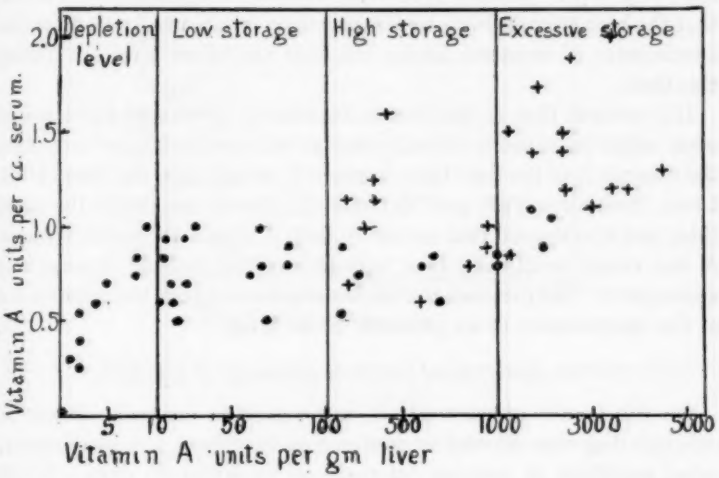

CHART 3. RELATIONSHIP BETWEEN THE CONCENTRATION OF VITAMIN A OF THE SERUM AND THAT OF THE LIVER

Note: The solid circles refer to rats in which the effect of recent ingestion of vitamin A has been excluded. As explained in the text these rats were (1) those receiving relatively small doses (less than 500 units a day) and (2) those that had received over 1,000 units per day but in which the administration of vitamin A had been discontinued at least three days before the animals were sacrificed. The crosses refer to rats receiving large doses of the vitamin (2,500 units (approximately) a day) up to within 2 days of the determination). In the latter group the high serum levels are probably due to the failure of more or less saturated tissues to remove vitamin A from the blood as fast as it comes. It should be emphasized that no animal was sacrificed within 24 hours of the last dose of vitamin A.

great rapidity when vitamin A ingestion is stopped and with it the blood level. Davies and Moore (18) found that about 90 per cent of the stored vitamin disappeared in about 4 weeks when pushed to extremely high levels. Lease and Steenbock (19) showed that the

rate of depletion was proportional to the amount present, but from their figures, as also those of Baumann, Riising and Steenbock (13), it is clear that the rate of depletion at levels represented by the figures reported here is not great enough to account for the differences between the two groups. Moreover, Brenner, Brookes and Roberts (16) found that the high storage levels were maintained for about a week after discontinuance of excessive intake but that the blood level fell during this time.

It is evident that as the liver concentration is reduced there comes what might be called a critical point at which with further reduction the vitamin A of the liver is no longer able to maintain the blood level. Lewis, Bodansky, Falk and McGuire (12) found essentially the same thing and also showed that on low vitamin A intake, the serum vitamin A was rasied practically to a normal level before liver storage was appreciable. The presence of this phenomenon suggests the importance of the maintenance of an adequate blood level.

3. Relation of blood level to development of deficiency

For this section of the work the rats placed on the basic vitamin A deficient diet were allowed to continue on it without any supplement, being sacrificed at varying intervals up to 40 to 50 days. In all instances included here, the mother had been given the deficient diet through the nursing period.

As may be seen from charts 4 and 5 both the vitamin A of the serum and that of the liver fell rapidly at first, reaching a point close to depletion by about the tenth day. Then the rate of fall was slower and complete disappearance from liver and blood was not reached until the 20th day. Thereafter in no case in which determinations were made was vitamin A found in the serum or liver, when the deficient diet was commenced at the time of weaning and continued without interruption to the time the rat was sacrificed.

Although complete disappearance of the vitamin from serum and liver occurred by the 20th day, the first "clinical" evidence of deficiency did not appear until the 28th day at the earliest, and usually somewhat later. The first sign was a ring of redness about the eyes, followed shortly by loss of appetite, snuffles and rapid development of keratomalacia. Loss of subcutaneous fat was thereafter rapid, and respira-

tory obstruction became more and more marked, the animals eventually dying from this cause if not sacrificed or given vitamin A. Often before death there was diarrhea. In 2 of the 30 litters studied, paralysis

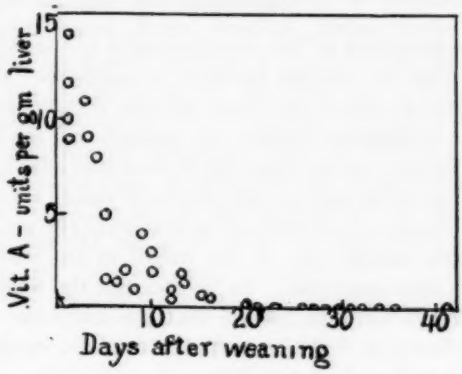

CHART 4. VITAMIN A OF LIVER DURING DEVELOPMENT OF DEFICIENCY IN YOUNG RATS

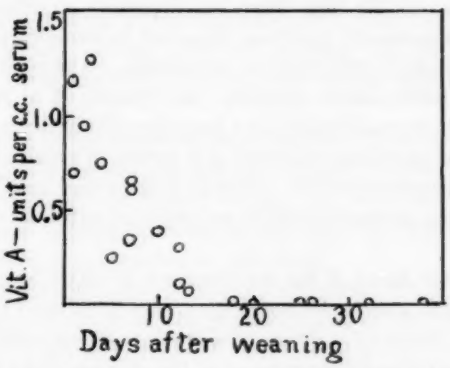

CHART 5. VITAMIN A OF SERUM DURING DEVELOPMENT OF DEFICIENCY IN YOUNG RATS

of the hind limbs was a prominent feature, although weakness was marked in all. Those rats that had developed paralysis recovered rapidly when vitamin A was administered.

The delay in the development of signs of deficiency has been commented on by others who have studied the problem (13, 20, 21). It will be discussed below.

DISCUSSION

The relative constancy of the vitamin A level in the rat under wide variations of intake and storage is worthy of emphasis. It is not that variations in serum level do not occur, but that they are so small compared with the variations of intake and storage. In the dog (15 and personal observation), on the other hand, the serum level is much more influenced by the intake, and serum vitamin A values are encountered 24 hours after vitamin A administration that would be seen in humans (5, personal observation) only at the height of the post-absorptive curve (4 hours after ingestion). In the rat and the human it seems likely that there is some mechanism that maintains the serum level within certain limits, as if there was danger from too much vitamin A in the serum as well as from too little.

The fact that the blood level is maintained at the expense of the stores in the liver, apparently assures the organism an adequate supply of vitamin up to the time when the depletion is virtually complete. The same phenomenon has been observed in humans, in the study of the development of deficiency in volunteers. Although carotene may be depleted with relative rapidity, the vitamin A of the serum may remain within normal limits for a long time (23 and personal observation). It has also been observed in a survey of children coming to an outpatient department that a diet had to be bad and bad for a long time before the serum vitamin A was reduced with any degree of regularity (3).

On the other hand, if the maintenance of blood level is significant because by this means the organism is protected until the last minute against the effects of a deficient diet, how do we explain the latent period between the disappearance of the vitamin from the serum and the development of signs of deficiency? In some of the rats of this series deficiency had not appeared as long as 5 weeks after depletion was complete, and in the rats studied by Horton, Murril and Curtis (21) the latent period amounted to as much as 8 weeks. Moreover, deficiency could be prevented by a daily administration of 2 units of

vitamin A a day (12, 22), an amount too low to cause the appearance of an appreciable amount in the blood.

The answer appears to be that what we consider the specific signs of deficiency are actually late signs and can be prevented by extraordinarily small doses of the vitamin. Before these changes take place, however, there must be changes of a metabolic nature which are a more delicate expression of the deficiency but less specific in their manifestations. Lewis and his co-workers (12) found that although they were able to prevent the specific signs of deficiency with the minimum dose of 2 units daily, the gain in weight did not become optimum until as much as 25–50 units a day were given. In the rats reported here, weight loss did not occur until the specific signs of deficiency were present, but there did occur changes which will be discussed in the next paper—namely a reduction in the blood lipids.

SUMMARY

Rats were studied with the object of learning something of the factors influencing the vitamin A of the blood serum, that might have a bearing on clinical interpretation. The following conclusions were reached:

1. The vitamin A of the serum is extraordinarily constant under wide variations in intake. When, however, excessive amounts of vitamin A are ingested the serum level 24 hours after the last ingestion tends to be higher.

2. The effect of recent ingestion in raising the serum vitamin A level is greater the higher the concentration of vitamin A in the liver. This is interpreted to mean that tissues already well filled are unable to remove the vitamin A from the blood as fast as it comes with the result that there is some accumulation in the blood.

3. Except for recent ingestion there is no evidence that the level of storage has any influence on the concentration in the serum, except that as vitamin A becomes depleted there comes a time when the vitamin A of the blood can no longer be maintained and the level falls abruptly.

4. When rats are placed on a deficient diet at the time of their weaning the vitamin A of liver and blood falls rapidly reaching depletion by the 20th day. Signs of deficiency, however, do not make their appearance for at least a week later and usually longer.

5. The changes that we recognize as vitamin A deficiency are late changes. It is felt, however, that there are probably other changes initiated by the lack of vitamin A, possibly of a metabolic nature, that precede the anatomical changes, and may even be the direct cause of them. Evidence for this point of view will be presented in the next paper.

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STUDIES IN VITAMIN A

Influence of Vitamin A on Serum Lipids of Normal and Deficient Rats

HUGH W. JOSEPHS

From the Harriet Lane Home of the Johns Hopkins Hospital and the Department of Pediatrics, Johns Hopkins University, Baltimore, Maryland

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The solubility of vitamin A in fat has led a number of investigators to attempt to find some relationship between vitamin A and lipid metabolism. Green (1), for instance, as the result of his observations thought that the loss of subcutaneous fat which occurred regularly in vitamin A deficient rats was no greater than in rats on a similarly restricted food intake. Sampson, Dennison, and Korenchewsky (2) claimed, on the other hand, that the decrease in food intake was insufficient to account for the failure of growth. In the end stages of deficiency they found some decrease in the absorption of fats, but Irwin, Steenbock and Kemmerer (3) showed that this decrease in fat absorption was not specific for vitamin A because it occurred in animals deficient in vitamin B, vitamin D and also in anemic animals fasted for a few days. In any case the amount of decrease in fat absorption was insufficient to account for the extreme loss of fat.

A number of observations suggested that fat might serve as a vehicle for the storage or transport of vitamin A. Dann (4) found that when the fat intake of pregnant rats was increased the young were born with somewhat greater stores of vitamin A. Thorbjarnarson and Drummond (5) claimed that conditions increasing liver fat also caused a greater amount of vitamin A to be stored, while choline diminished the amount of vitamin A as well as the fat of the liver. Josephs (6) showed in clinical material that there was a rather broad relationship between total lipid and vitamin A of the serum which he was inclined to attribute to the possibility that the fat of the blood served as a vehicle for vitamin A. It was also found that when serum lipids were low, there was little if any rise in the serum vitamin A after ingestion of large amounts, whereas a significant rise in the postabsorptive

vitamin A curve occurred only when the serum lipid levels were at least within normal limits.

Evidence that vitamin A specifically influences the metabolism of lipids is furnished by some German work. It was found by a number of workers (7, 8, 9, 10, 11) that when enormous doses of vitamin A were given to animals, there occurred an increase in the fat deposits in the cells of the reticuloendothelial system, and Collazo and Rodriguez (9) found similarities between hypervitaminosis A in the rat and Schüller-Christian's syndrome in the human. Jusatz (12) found in humans that the administration of a vitamin A concentrate caused a rise in the cholesterol and neutral fat of the serum. He also found (13) that in rabbits previously rendered deficient in vitamin A, administration of vitamin A caused a marked increase in the serum cholesterol and neutral fat. Lasch (14) confirmed this finding in normal humans. Wendt (11, 15) also found the same effect from large doses of vitamin A in rabbits, rats, and humans, but was unable to repeat the observations in dogs. He observed, moreover, that when the high vitamin A dosage was maintained over a long time, the normal values were gradually resumed. From this he concluded that there was a mechanism which compensated for the effect of overdosage with vitamin A (11).

The work reported here is an attempt to confirm and extend these observations suggesting that vitamin A exerts a specific effect on lipid metabolism. This possibility receives added interest from the suggestion made in the preceding paper regarding the "latent" period. This was the period between the complete disappearance of vitamin A from the blood and liver, and the appearance of signs of deficiency sometimes as much as three to four weeks later. It was suggested that the latent period was not due merely to the gradual development of anatomical lesions to a point where they become manifest, but might be a interval during which certain metabolic changes were developing which we had not yet learned to recognize. The problem has an important clinical bearing, for extremely low blood values may be present in infancy especially in severe infections, gastrointestinal disturbances and disease of the liver, but anatomical evidence of vitamin A deficiency is a comparatively rare finding at autopsy.

The plan was to study the blood and liver lipids during the develop-

ment of vitamin A deficiency, and to determine the relation to disappearance of the vitamin from the blood and liver and to the appearance of manifest signs of deficiency. Then the surviving animals were to receive large daily doses of vitamin A and the effect of this on the lipid levels of the blood and liver, as well as on the vitamin A of the twenty-four hour blood sample was to be determined. This was to be compared with the effect of equal doses of vitamin A on non-deficient animals raised on the same basic diet to which sufficient vitamin A had been added to prevent deficiency.

METHODS

The method of sacrificing the animals and obtaining the blood and liver specimens was the same as given in the preceding article. The methods for determining the vitamin A and total lipids of the serum have been reported previously (6). The lipids of the liver were determined as follows: a weighed sample was ground with sand in a small mortar with a few cubic centimeters of Bloor's solution (3 parts 95 per cent alcohol, 1 part ether) and transferred with further additions of Bloor's solution to a small flask, 15 cc. being used in all. The procedure then followed is that for blood as previously described. The basic vitamin A deficient diet, as well as the method of administering the measured doses of vitamin A, was described in the preceding paper.

RESULTS

1. Total lipid of serum during development of deficiency

The rats used in this part of the study comprise two groups: (a) Rats placed on the basic deficient diet at the time of weaning (22 days) and given a supplement of vitamin A; (b) Rats placed on the basic diet and given no supplement. These rats were the same as those used in the preceding study to determine the relation of vitamin A intake to serum vitamin A and the relation of serum vitamin A to the development of deficiency.

As reported in the previous paper, the rats on the deficient diet showed considerable variation in the speed with which they developed signs of deficiency. None showed any manifestation before the twenty-eighth day on the diet, while occasionally a rat went for as long as ten weeks without any evidence of deficiency. Most of them, however, manifested deficiency between the fifth and seventh weeks. On the

other hand, the vitamin A had disappeared from the blood and liver in all the rats by the twentieth day.

In chart 1 are plotted the values for serum lipids of rats receiving vitamin A supplement during growth from the time they were weaned (solid circles). Since a preliminary analysis showed that the size of the vitamin A dose made no difference in the lipid values, no distinction is made in the chart between rats receiving large and those receiving small doses. It may be seen that the normal limits of variation lie between 3 and 4.5 milligrams per cc. In the same chart are plotted

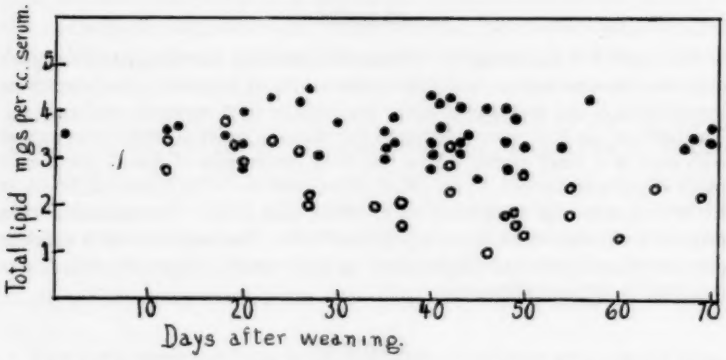


CHART 1. COURSE OF SERUM LIPIDS IN NORMAL RATS AND THOSE RECEIVING DEFICIENT DIET DURING PERIOD OF DEVELOPMENT

Note: The solid circles represent figures from normal animals; the hollow circles figures from animals on diet deficient in vitamin A.

the values from rats placed on a deficient diet from the time they were weaned (hollow circles). Here it is evident that there is a reduction of lipid values which becomes significant after about three weeks on the deficient diet.

There was no clear relationship between the fall in lipid levels and the development of manifestations of deficiency, except the general relationship that the tendency to low serum lipid values had reached its greatest development at about the time the majority of the animals were showing signs of deficiency. However, a few animals with severe manifestations showed no reduction in lipid level, while others without manifestations of deficiency showed a well marked reduction.

The possibility that the reduction in serum lipid was dependent on a reduced level of food intake was investigated. Two pairs of rats were placed on the deficient diet and the food intake of each pair carefully weighed each day. Two pairs of control rats were placed on the same diet to which a few drops of halibut-liver oil had been added. The amount of food which each pair received was the same as that consumed by the corresponding deficient pair during the preceding twenty-four hours. After forty days all eight rats were sacrificed. The rats on the deficient diet all showed signs of deficiency (sore eyes, bloody snuffles) and in every case the serum lipid level lay below 2 milligrams per cc. The control rats were normal except for being slightly underweight, and the serum lipids varied between 3.1 and 4.3 milligrams per cc. In spite of the fact that the food intake was the same, the weight of the deficient rats was considerably below that of the control rats, thus confirming the earlier finding of Sampson, Dennison, and Korenchewski (2).

The next question was whether deficiency caused a failure of fat absorption sufficient to account for the loss of body fat and the lowering of the blood lipids. Figures in the literature give indications on this point. Sampson, Dennison, and Korenchewski (2) showed that there was no significant disturbance of either nitrogen or fat absorption until signs of deficiency had become marked. Irwin, Steenbock, and Kemmerer (3) found also that there was diminished absorption of fat in animals showing signs of deficiency but since it was not marked, and was present also in animals showing loss of weight from other causes than vitamin A deficiency, they were inclined to attribute it to the nutritional state of the animal rather than to a specific effect of vitamin A deficiency.

Since the loss of body fat is so clearly associated with signs of deficiency, when absorption is shown to be reduced, it remains quite possible that the reduction in absorption is a factor in the loss of body fat whether or not it is specifically due to the vitamin A deficiency.[1] Reduction in absorption however, cannot be the cause of the diminution in the blood lipid level; not only has this been found in the period before the development of deficiency to a point where failure of absorp-

[1] Another factor, not related to absorption, may be an increase in metabolic rate in deficient animals reported by a number of workers (2, 16, 17).

tion becomes of importance but it has also been observed in animals in which appetite was preserved and in which little or no loss of weight had occurred. From these considerations it seems evident that the reduction in serum lipids is a specific pathological effect of an actual internal deficiency of vitamin A.

Here then is a metabolic change produced by vitamin A deficiency occurring during a time when the rat gives no external evidence of deficiency. As was pointed out above there is no clear relationship between the reduction in lipid level and the development of the manifestations of deficiency that depend on anatomical change. This is not to claim that such anatomical changes may not be present and that finer histological methods may not uncover them much closer to the time of the disappearance of vitamin A from the body. However the fact that the outward manifestations of deficiency, including loss of weight, appear rather abruptly and once present advance with great rapidity, suggests that the period of latency is one in which certain metabolic changes are evolving and that the development of the characteristic anatomical lesions are dependent on the presence of these metabolic changes. The problem of the relationship of the metabolic changes to anatomical lesions will probably not be solved until we have means, whether histological or chemical, of studying the metabolic activity of the individual cell.

The suggestion that the anatomical lesions are dependent on metabolic changes occurring during the latent period is in line with that made by Baumann, Riising and Steenbock (18), in discussing this period of "latency," that vitamin A might have a role in the prevention of "toxic" changes, which in absence of the vitamin would go on to cause the characteristic lesions. The diminution in serum lipids suggests that the role played by vitamin A is not that of a "detoxicant," but rather that it has an important function as a regulator of lipid metabolism.

2. Total lipid of the liver during development of deficiency

A. Twenty-five control rats were studied. These had received vitamin A in amounts varying from about 10 units a day up to about 2000 units. In twenty-two of these twenty-five, the liver lipids varied between 5 and 7.5 per cent. Only one figure lay below 5 per cent and the highest was 10.5 per cent. There was no demonstrable effect from

age or vitamin A dosage, but the number of determinations is too small for certainty regarding these points.

B. Seventeen rats were examined at various periods during the development of deficiency. Of the seventeen, thirteen had values for liver lipids that varied between 4 and 6 per cent. Four of the values were above 6 per cent; two of these occurred within the first twenty days, and two after the forty-fifth day. In general the levels in the first thirty days tended to be somewhat higher than later.

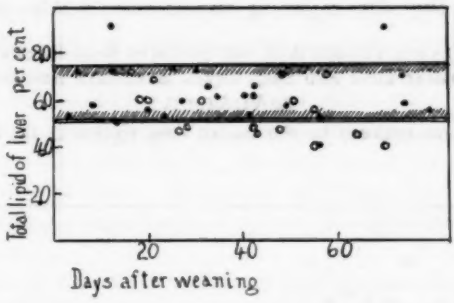


CHART 2. COURSE OF LIVER LIPIDS IN NORMAL RATS AND THOSE RECEIVING DEFICIENT DIET DURING PERIOD OF DEVELOPMENT

Note: The solid circles represent figures from normal animals; the hollow circles figures from animals on a diet deficient in vitamin A.

The liver lipids then appear to be gradually reduced during the maintenance of a deficient diet, but do not take part in the extreme reduction characteristic of the other fat depots of the body. The significance of these changes in level of liver *lipid* with deficiency is not known.

3. Vitamin A and lipids of the serum after administration of vitamin A

In these experiments three series of rats were used.

A. Rats were placed on a vitamin A deficient diet when they were weaned at twenty-two days and kept on the diet until signs of deficiency were present in at least the majority of the litter. When this point was reached (usually 35–45 days) they were started on the administra-

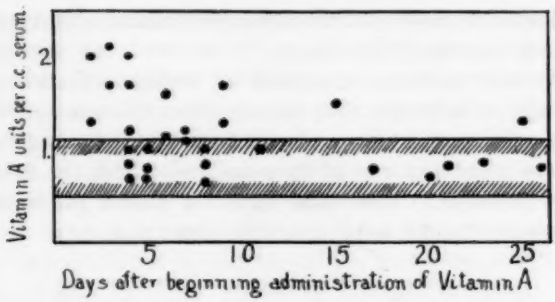


CHART 3. VALUES FOR VITAMIN A OF THE SERUM OF RATS RAISED 5-6 WEEKS ON NORMAL DIET AND THEN GIVEN EXCESSIVE AMOUNTS OF VITAMIN A

Note: The area enclosed by the shaded lines represents the zone of normal values.

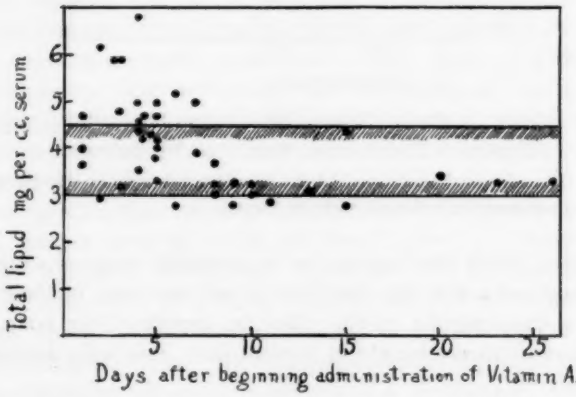


CHART 4. VALUES FOR TOTAL LIPID OF THE SERUM OF RATS RAISED 5-6 WEEKS ON NORMAL DIET AND THEN GIVEN EXCESSIVE AMOUNTS OF VITAMIN A

Note: The space enclosed by shaded lines represents the zone of normal values as determined in chart 1.

tion from a syringe of 0.1 cc. daily of undiluted halibut-liver oil. The rats were sacrificed after varying intervals, each point on the charts representing one individual.

B. Rats were raised for the same length of time (35–45 days) on the same basic diet as in the case of series A, but a few drops of halibut-liver oil were added to the diet to prevent deficiency. At the end of this preliminary period, these rats were started on the administration of undiluted halibut liver oil exactly as in the case of series A.

C. The preliminary period was carried out as in the case of series A. These rats were then given halibut-liver oil that had been diluted with four times the amount of corn oil. 0.1 cc. was administered daily so that they received the same amount of oil as did the rats of series A but one-fifth the amount of vitamin A.

When large doses of vitamin A were given to non-deficient animals (series B) (charts 3 and 4), there occurred a rise in the serum lipids to a point well above the normal range within the first two or three days followed by a rapid fall to normal, the rise and subsequent fall lasting about six to seven days.[2] The vitamin A of the serum paralleled the curve of total lipids. Thereafter both total lipids and vitamin A remained within normal limits, in spite of the continued administration of vitamin A.

The results in the case of vitamin A in large doses given to previously deficient animals (series A) are shown in charts 5 and 6. From these it is evident that a tendency to a rise in vitamin A and total lipids of the serum was far greater in the deficient than in the normal animal and lasted a much longer time.

When smaller doses of vitamin A were given to deficient animals (series C) the vitamin A and total lipid of the serum returned to the "normal" level with no tendency to rise above the "normal." This series is important for control, for (1) the amount of oil given to these animals with the vitamin A was the same as in the other two groups and (2) the rate at which these animals recovered from their deficiency,

[2] In speaking of a rise and subsequent fall, it must of course be understood that we cannot speak of the occurrence in an individual animal, but only as a tendency represented by the points in the charts. Not all the points obtained at the time when the "curve" was at its apex will be found above the normal range. But this as well as the fact that in some instances probably no rise whatever occurred is no reason to assume that the rise when it did occur was no more than chance. Such variability in individual response may indicate differences in the ability of various animals to compensate for the sudden introduction of large amounts of vitamin A as will be discussed below.

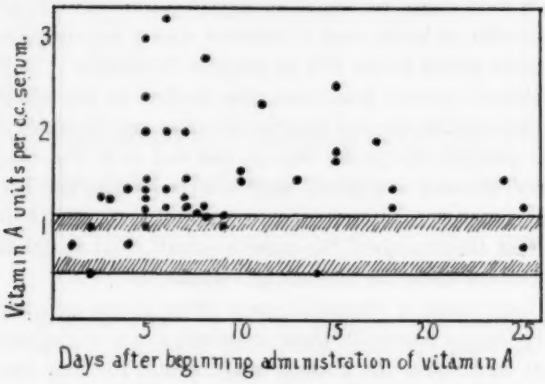


CHART 5. VALUES FOR VITAMIN A OF THE SERUM OF RATS RENDERED DEFICIENT IN VITAMIN A AND THEN GIVEN EXCESSIVE AMOUNTS OF THE VITAMIN

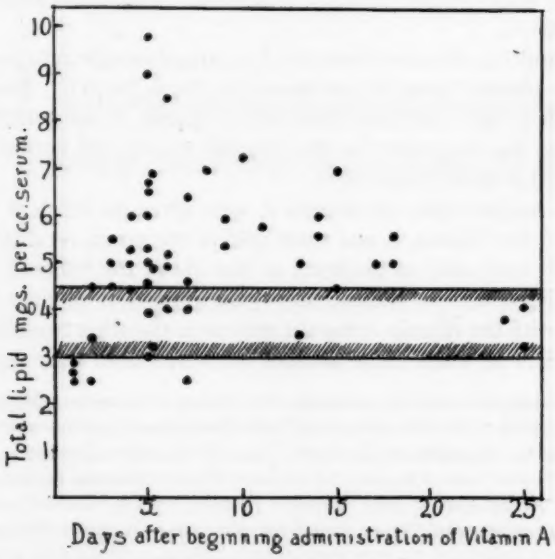


CHART 6. VALUES FOR TOTAL LIPID OF THE SERUM OF RATS RENDERED DEFICIENT IN VITAMIN A AND THEN GIVEN EXCESSIVE AMOUNTS OF THE VITAMIN

gained weight and restored their fat depots was the same as in the case of the deficient animals of group A.

To summarize the results obtained in this series of observations: When vitamin A was *abruptly* introduced in *excessive* amounts, there occurred a parallel rise in vitamin A and total lipids of the serum. The rise was much greater in the deficient than in the normal animals. It was temporary but lasted a much longer time in the deficient than in the normal animals. When a smaller dose of vitamin A was used this phenomenon was not observed though the deficient rats on the smaller dose recovered from their deficiency as rapidly as the rats on the excessive doses.

4. Rate of deposit of vitamin A and lipid in livers of normal and deficient animals

The rats used were those of series A and B and the purpose was to determine whether deficiency brought about any disturbance in the ability to store vitamin A. In chart 7, the total amount of vitamin A in the liver is plotted. In this there is seen to be a slight lag in deposit of vitamin A in the deficient animals. This has been found by others (18) and interpreted to mean poor absorption of vitamin A caused by the results of deficiency. It seems just as likely that the small size of the liver is the limiting factor, and that with the rapid increase in size which occurs after a day or two, the liver of the deficient animal rapidly catches up with that of the non-deficient in its ability to store vitamin A.

From chart 8, it may be seen 1) that there was no difference in the rate at which the concentration of vitamin A increased for normal and deficient rats, and 2) that during the period of observation the concentration had not become excessive. In other words deficiency causes no disturbance in the ability of the liver to store vitamin A, and one therefore cannot explain the high levels of serum vitamin A on this basis. In fact the high levels in previously deficient rats occurred at a time when the total amount in the liver was increasing most rapidly.

The increase in total lipids of the liver was studied in twenty cases. It may be recalled that in non-deficient animals the liver lipids varied between 5 and 8 per cent while in deficient animals the variation was from 4 to 6 per cent. In series B (control) there was no change in

liver lipids (5 cases). In series A (deficient animals) within five days the liver lipid values had risen to between 6 and 8 per cent. Deficiency, therefore, causes no impairment of the ability of the liver to store lipids.

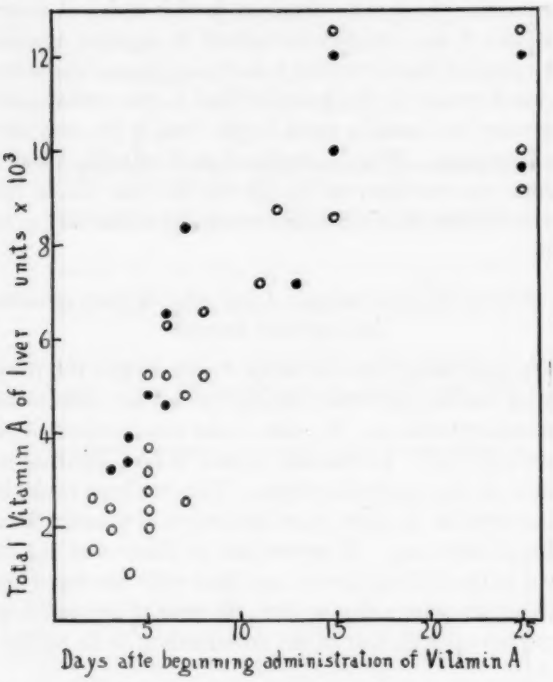


CHART 7. INCREASE OF TOTAL AMOUNT OF VITAMIN A IN LIVER DURING ADMINISTRATION OF LARGE AMOUNTS OF VITAMIN A

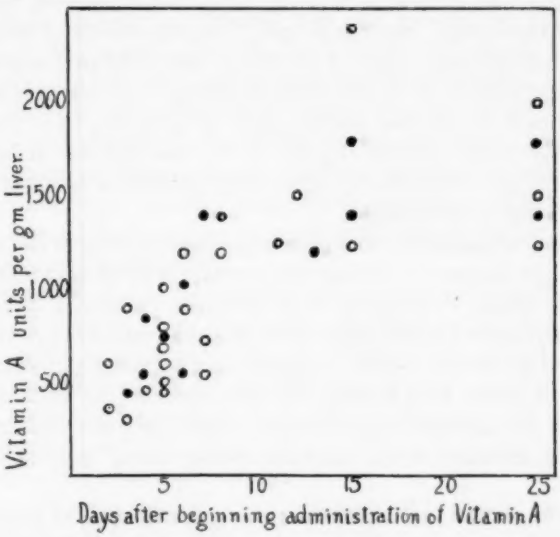
Note: The solid circles represent figures from normal rats; the hollow circles figures from rats previously rendered deficient.

DISCUSSION

The effect of a high vitamin A intake on the level of serum vitamin A was discussed in the preceding article. It was shown there that the effect of excessive doses in raising the serum vitamin A in a specimen taken after twenty-four hours was probably due to a lag in the removal of vitamin from the blood by the liver, but that only when the stores in the liver were excessive did the serum values tend to reach relatively high levels. This was interpreted as a type of "saturation" phenomenon.

In the rats of the present study given large doses of vitamin A abruptly, the curve of the vitamin A of the serum rose to a peak that was higher than that reached in the rats discussed in the preceding

paper. This was especially true of the previously deficient animals. If "saturation" played a part in causing the rise in serum levels, this might occur in one of two ways: 1) as in rats of the preceding paper, as the result of the filling of the stores to a point where they could no longer remove vitamin A from the blood as rapidly as it came; 2) as the result of an impairment of the ability of the liver to take up the vitamin when suddenly presented with a large amount, until it had had time to become adjusted to the higher level of intake. This second possibility is one that becomes especially plausible if one adds the



GHART 8. INCREASE OF VITAMIN A CONCENTRATION IN LIVER DURING ADMINISTRATION OF LARGE AMOUNTS OF VITAMIN A

Note: The solid circles represent figures from normal animals; the hollow circles figures from animals previously deficient.

reasonable assumption that the ability to make the adjustment to the increased vitamin A intake is further impaired as the result of previous deficiency.

Both these possibilities can, however, be eliminated. In the first place at the time of the peak of the serum vitamin A values, the liver vitamin A store had not yet reached the level of "saturation" as observed in the previous paper, while by the time this point was reached, the serum vitamin levels were returning to "normal" figures. In the second place, at the time of the high serum levels, the rate of deposition of the vitamin A in the liver was found to be at its maximum, and it was not found that the previous deficiency had caused any impairment in the rate at which liver stored the vitamin. It seems more likely therefore, that we have an example of the possibility discussed in the intro-

duction; namely, that the blood lipids act as a vehicle for vitamin A, holding it in the blood so that for a time it may tend to accumulate. There is no evidence that the rise in vitamin A serves any useful purpose. The deficient animals had recovered from their deficiency some days before the rise in blood vitamin A had reached its height.

While we tend to dismiss the rise in serum vitamin A as a secondary phenomenon of interest in a study of factors influencing the serum levels of the vitamin, the rise in lipids is an occurrence of the greatest importance, the explanation of which may lead to the proper evaluation of the part played by vitamin A in physiology. As we have seen, the fall in lipids during the development of deficiency was attributed specifically to the lack of vitamin A; so also the rise in lipids is a specific effect of vitamin A. One other possibility however, should be mentioned to be excluded.

It is not unreasonable to suppose that tissues storing or metabolizing lipids might become so adjusted to a certain level of lipid intake, that when the intake is increased there would be required a certain time for a readjustment to the higher level, and that tissues deficient in their lipid content would require a longer time to make the adjustment. The result would be a period, relatively short in the normal animal, longer in the deficient animal during which the extra lipid absorbed from the intestine would circulate before being deposited in the tissues.

Actually in the case of the normal animals of these experiments the extra oil could only come from that used as a vehicle for the vitamin A, amounting to .1 cc. per day. This amount is certainly not likely to put any strain on the storage capacity of the normal rat. In the case of the deficient rats, the additional lipid was greater for it included not only the vitamin A vehicle but also the increase in intake and absorption over that of the deficient animal. However, the rapidity with which the fat stores were replenished makes it evident that there was no disturbance in fat deposition. Moreover, the results of giving the diluted vitamin (series C) indicate that the deficient animal was able to take care of the extra fat without rise in the serum lipids. They also indicate that the lipid rise was specifically due to an excessive amount of vitamin A and not a mere accompaniment of the recovery process in which rise in serum lipids would be looked upon as a reaction designed to furnish increased transport.

As Wendt (11) pointed out, the rise in lipids and the subsequent return to normal values while excessive vitamin A administration is being continued argues for the presence of a mechanism by which the body adjusts itself to, or, if one wishes, protects itself against, the excessive administration. The fact that toxic effects are produced only with enormous overdosages means that the body is able to adjust itself over a wide range. One factor in this adjustment has been suggested by Davies and Moore (19) who pointed out that toxic effects are not determined by the total amount of vitamin A in the body, but by an intake so excessive that the liver is unable to remove the vitamin A from the circulation rapidly enought to prevent harm. In other words, vitamin A deposited in the liver appears to be inactive. A second factor appears to be the rapidity with which excessive stores disappear when high vitamin A dosage is discontinued. What happens to this vitamin, whether it is destroyed *in situ* or excreted in some unrecognized form, is not known.

The observations reported here as well as elsewhere suggest that one function of vitamin A is concerned with lipid metabolism. They suggest further that the body tends to protect itself against a disturbance in the stability of the blood lipids; that this protective mechanism may be overcome temporarily by a sudden flooding of the organism with vitamin A, and that it may be impaired in its ability to respond as a result of vitamin A deficiency.

Our knowledge is not sufficient yet to attempt to specify the manner in which the body compensates for the effect of an increase in the physiological activity of vitamin A brought about by the increased intake, whether by rendering the vitamin A inactive or by opposing its activity by the development of some antagonistic process. There is a large body of literature from German sources that indicates that vitamin A and the thyroid are antagonistic in a physiological sense.[3] Whether or not a compensatory reaction that involves changes in

[3] This literature is not reviewed here, since it would be unprofitable to enter on a discussion of the subject without more facts at our disposal. However, the possibility of explaining the recorded facts by a thyroid-vitamin A antagonism would certainly occur to anyone familiar with the subject. The literature on the subject is well covered in S. W. Clausen's review of vitamin A in the series of articles on the vitamins recently published by the American Medical Association (21).

thyroid activity is present in the rats studied here is for the future to decide.

Apart from its possible use in investigating the physiological action of vitamin A, its effect on serum lipids has perhaps a more immediate practical use as a basis for a possible physiological test of deficiency in the human. This, of course, could depend on the sensitivity of the lipid response to the administration of vitamin A which was found to be so much greater in the previously deficient animal. Preliminary observations on infants indicate that such a test may have some value. Moreover, Steininger, Roberts, and Brenner (20) found, when they gave vitamin A in relatively small doses to deficient volunteers, that a rise occurred in the vitamin A of the serum to a point above that present on a previous normal diet and lasting a considerable time. They were at a loss to expain it. The reaction, however, is not unlike that in the deficient rats reported here, except that the dose that elicited it was relatively much smaller.

SUMMARY

The effect of vitamin A deficiency and vitamin A administration on serum lipids was studied in rats. The following conclusions were reached:

1. The development of vitamin A deficiency was associated with a gradual fall in serum lipid levels. This fall took place during the period of "latency" between the disappearance of vitamin A from liver and blood and appearance of the manifestations of deficiency.

2. During the development of deficiency the lipids of the liver tended to fall slightly. This was in contrast to the depletion of the fat depots elsewhere in the body.

3. When non-deficient rats were examined after beginning the daily administration of excessive amounts of vitamin A it was found that the serum lipid level and the serum vitamin A concentration both tended to rise above the normal range, then returned to normal after a few days.

4. When rats previously rendered deficient were given daily excessive amounts of vitamin A, the subsequent tendency to rise in serum lipid and vitamin A levels was much more pronounced than in the normal animals and lasted often for more than two weeks.

5. Previous deficiency caused no disturbance in the ability of the

rat to store vitamin A or lipids in the liver, as soon as vitamin A was given and the animal started to eat.

6. It is believed from the evidence presented that the reduction in serum lipids was due specificially to the vitamin A deficiency and not to the nutritional state of the animal, the loss of appetite or failure to absorb fats.

7. The rise in lipids is attributed to a specific effect of vitamin A. To elicit it the vitamin must be given abruptly and in excessive amounts. The return to normal is thought to be due to a compensatory reaction, the efficacy of which is impaired by previous deficiency.

8. Possible application of the phenomenon of the rise in lipids after vitamin A to the problem of the determination of vitamin A deficiency in the human is suggested.

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AN EVALUATION OF PROGESTERONE THERAPY IN THE TREATMENT OF ENDOMETRIAL HYPERPLASIA

G. E. SEEGAR JONES, M.D., AND R. W. TE LINDE, M.D.

Department of Gynecology, Johns Hopkins Hospital and University, Baltimore, Maryland

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Functional uterine bleeding is a term generally applied to those cases of abnormal uterine bleeding in which no neoplastic or inflammatory process can be found in the uterus to account for the condition. It was early recognized that such bleeding had as a background a disturbance of the endocrine glands. Intelligent treament of functional uterine bleeding would therefore require an exact knowledge of this endocrine background as well as a thorough understanding of the physiology of menstruation. Such knowledge is at present very inexact.

The pathological picture of the endometrium in cases of functional uterine bleeding has been extensively studied. At one time it was thought by many gynecologists that functional uterine bleeding was invariably associated with endometrial hyperplasia. This point of view is no longer tenable, for there is now overwhelming evidence which demonstrates that any type of endometrium may be found with this clinical symptomatology (1, 2, 3, 4, 5, 6). Jones (7), in earlier studies from this department, found that endometrial hyperplasia was present in 63 per cent, secretory endometrium in 17 per cent, and interval, post-menstrual or atrophic endometrium in the remaining 20 per cent of the cases.

From the standpoint of symptomatology and etiology, however, it is apparent that the most homogenous group with functional uterine bleeding is made up of those cases associated with the pathological pattern of endometrial hyperplasia. In the earlier literature it was generally accepted that in this group the fundamental hormone abnormality was a state of hyperestrogenism. That such a simple state of affairs does not always exist in the human is evident from the fact that it is not possible in every case to demonstrate in the urine or blood an excess of the estrogenic hormones. Furthermore, Burch (8)

has offered abundant evidence from animal experiments to indicate that endometrial hyperplasia may result from various degrees of ovarian insufficiency. Such evidence seems to demonstrate that the endocrine background which results in bleeding from endometrial hyperplasia is probably variable. Consequently any method of therapy which attempts to adjust quantitatively a presumed hormonal imbalance is doomed to failure in a certain percentage of cases.

Schröder (9) and Meyer (10), working independently, and Novak and Martzloff (11) found by examination of the ovaries in cases of endometrial hyperplasia that a recent corpus luteum could almost never be demonstrated. It is reasonable to infer from this finding that whatever the relation of estrogen to endometrial hyperplasia there must be an absence of progesterone. That such is the case has been borne out by the failure of Browne (12) and others (13) to demonstrate any pregnanediol in the urine of patients with endometrial hyperplasia. Hamblen (14) has reported the presence of pregnanediol under such circumstances but for technical reasons there is some doubt as to the identification of his product.

Before progesterone became available for clinical use it was generally expected that this hormone would be a panacea in the treatment of endometrial hyperplasia. However, about ten years have now elapsed since the introduction of progesterone and the previous expectations have not been fulfilled as judged by most reports. Nevertheless, it is our opinion that the corpus luteum hormone is extremely useful in the treatment of endometrial hyperplasia if it is properly administered and its limitations are appreciated. The rationale for progesterone therapy is clear in that it supplies an evident deficiency. The apparent poor therapeutic results which are obtained can generally be explained on the basis of one or two conditions. Either the proper dosage is not given or the physiological role of progesterone in normal menstruation is not understood. Progesterone is not useful in checking immediate hemorrhage. In the normal cycle it is the withdrawal of progesterone which initiates within forty-eight to seventy-two hours the onset of menstruation. The withdrawal of the corpus luteum hormone may therefore be said to produce a chemical curettage and thus it is not to be expected that the bleeding will be controlled short of four days following progesterone withdrawal. It must be

fully realized that the use of progesterone is a substitution therapy and may consequently need to be carried out over a long period of time.

It is the purpose of the present paper to describe our experience with a method of progesterone therapy which has now been used over a sufficient period to allow observation of some patients for as long as five years. Of the entire group of patients with functional uterine bleeding only those with endometrial hyperplasia are herewith reported. As stated above this group represents only cases of functional bleeding in which progesterone deficiency is an etiological factor and is the only group in which progesterone therapy could be expected to be therapeutically effective.

RESULTS OF THERAPY BEFORE THE USE OF PROGESTERONE

The evaluation of any method of therapy must take cognizance of the natural history of the disease being treated. Endometrial hyperplasia has long been known to exhibit spontaneous remissions. In specifically evaluating any hormone therapy in this disease it is also important to know the results of the simple surgical procedure of curettage. Next it is necessary to know how many patients are refractive to repeated therapeutic curettages and require more radical procedures, such as hysterectomy or irradiation therapy. In order to answer these questions and obtain a proper background for an evaluation of the results of progesterone therapy we have studied 111 cases of endometrial hyperplasia treated in this clinic prior to the advent of endocrine therapy. The results of this study are presented in Table I.

The cases have been arbitrarily divided into two groups, those above the age of thirty-five and those below. This has been done because the problems which arise in these two age groups are quite different. In those patients above the age of thirty-five years there are frequently other associated lesions such as myomata, adenomyosis, polyps of the cervix and endometrium and vaginal relaxation requiring surgical intervention. In those cases uncomplicated by such lesions, the patients are nevertheless at an age where the conservation of ovarian function is no longer such a serious consideration and therefore irradiation therapy is a simple and relatively satisfactory method of treatment. However, the discomfort and occasional complete disability that follow the precipitation of a premature menopause are

not to be considered lightly. Of the forty-eight cases in this age group, twenty-two were relieved of symptoms by a single curettage; three patients had repeated curettages, while twenty-three required more radical treatment; of these twenty-three cases in fourteen there was associated some other lesion. The use of irradiation therapy or surgery in this group seems still to be the most practical procedure and can be employed satisfactorily when there is any question as to the desirability of subjecting a patient to the prolonged period of observation which is necessary with hormone treatment.

It is in those patients below the age of thirty-five years in which there is the greatest need for hormone therapy. However, an analysis of this group also indicates that a simple dilatation and curettage is

TABLE I

The results of therapy in 111 cases of endometrial hyperplasia before the use of progesterone

NO. OF CASES	AGE	THERAPY	RESULTS
25	Under 35	Single D & C	Satisfactory
24	Under 35	Multiple D & C	Satisfactory (3 pregnancies)
14	Under 35	Radical procedures (hysterectomy or irradiation)	Unsatisfactory
22	Over 35	Single D & C	Satisfactory
3	Over 35	Multiple D & C	Satisfactory
23	Over 35	Radical procedures	Unsatisfactory

therapeutically valuable, as twenty-five out of sixty-three cases required only a single curettage for the relief of symptoms. This figure indicates that it is by no means necessary to begin hormone treatment as soon as the diagnosis of endometrial hyperplasia is made. Because of this favorable group it is also our opinion that the first curettage should be a thorough one, made as a therapeutic procedure under anesthesia. Endometrial biopsy is an important diagnostic aid, but the almost universal practice of resorting to an office biopsy followed by early hormone therapy in suspected cases of functional uterine bleeding is often unnecessary endocrinological meddling. The most serious problem concerns the remaining thirty-eight patients in whom a recurrence of bleeding followed, at varying intervals, the initial curettage. It is this group, and only this one, in which we now

feel that hormone therapy is necessary. The majority of cases treated with progesterone, to be reported here in detail, belong to this group. These patients offer the serious therapeutic problems as they represent the failures of other methods of treatment. Such patients comprise 40 per cent of the total number of cases of endometrial hyperplasia. In this group repeated curettages were performed in 24 instances. That even this tedious method may tide the patient along until somehow the hormone balance is reestablished is indicated by the occurrence of three subsequent pregnancies in this group. Fourteen of the thirty-eight patients had such difficulty following one or more curettages that hysterectomy or irradiation therapy was necessary. We may comment at this point that during the last five years under progesterone therapy no case in the age group of less than thirty-five was treated in this radical manner because of intractable bleeding. In all fairness it must be said that under these circumstances irradiation, either with X-ray or radium, sometimes effects a brilliant result by inducing a temporary amenorrhea which is followed by regular periods and occasionally pregnancy. There is, however, always the risk of a permanent cessation of menstruation, and for this reason even light irradiation therapy must be considered as radical treatment.

Such is the background against which the efficacy of hormone therapy must be considered.

RESULTS OF PROGESTERONE THERAPY

The cases which have been treated with progesterone number twenty-eight and with a few exceptions fall into the group in which it was undesirable to bring on the menopause because of youth or hope for a family, or both, and in whom one curettage was followed by further profuse bleeding. With one exception the results of therapy have been satisfactory in all cases (Table II).

The plan of treatment has been much the same for each case with certain minor individual variations which must be reviewed in the case reports. Before the initial treatment a thorough curettage under anesthesia was performed. The patient was then observed over an interval of one to three months and if no profuse or prolonged bleeding occurred no further therapy was instituted. If, however,

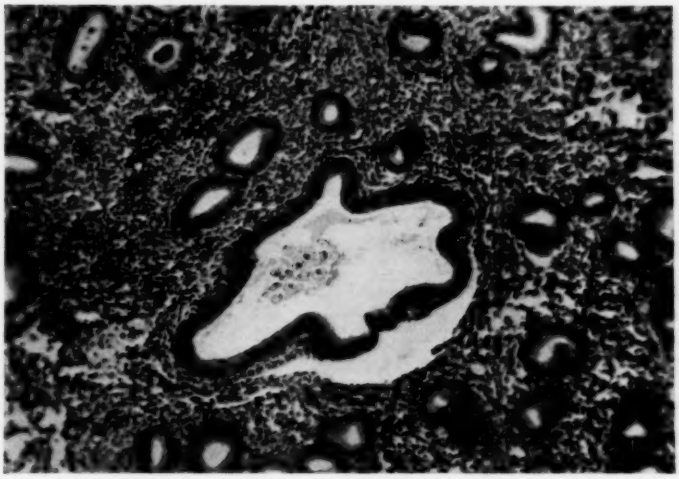


FIG. 1. A. Curettings obtained from case 10 before progesterone therapy. Typical endometrial hyperplasia. Photomicrograph. Low power.

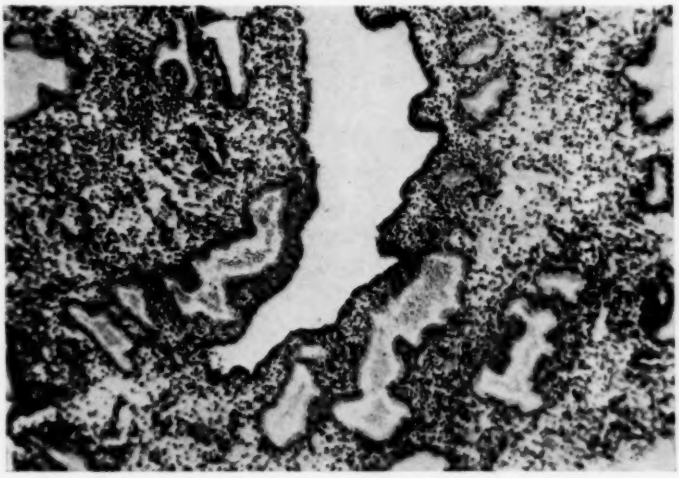


FIG. 1. B. Curettings from same patient after she had received 420 mgm. of pregneninolone over a period of 7 days. There is a well developed secretory endometrium. Photomicrograph. Low power.

a period of bleeding lasted over eight days and was excessive in amount, a suction biopsy was performed and progesterone therapy was begun. The patient was instructed to rest in bed on the supposition that her bleeding would not cease until four or five days after cessation of medication. During the first few years progesterone was given parenterally; 5 mgm. a day over a ten day period was given during the first month. The following month the dosage remained the same but the period of administration was shortened to seven days, beginning nine days before the calculated date of the next expected menses or twenty-nine days after the beginning of the first progesterone injections. The third month the injection period was again

TABLE II

The results of progesterogenic therapy in endometrial hyperplasia in 28 cases failing to respond to curettage

NO. OF CASES	THERAPY	RESULTS OF THERAPY
14	Single course of progesterone	Well, 9 cases. Amenorrhea and irregular menses, 5 cases
9	Repeated courses of progesterone	Well, 7 cases (4 still under therapy). Amenorrhea or irregular menses, 2 cases
3	Still under initial therapy	All well at present
2	Hysterectomy	(Operation done in one case because of salpingitis)

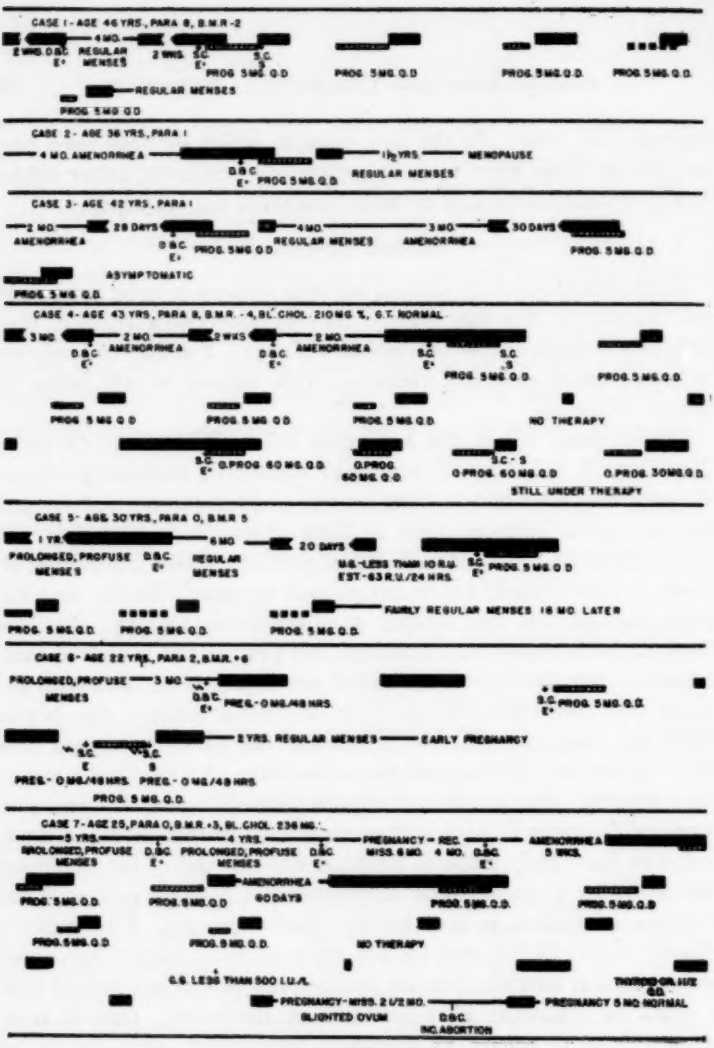
shortened, this time to four days beginning six days before the expected menses. When oral progestogen, pregneninolone, was first used the original cases were given 60 mgm. a day, in 10 mgm. tablets, over a period of seven days. This dosage was reduced to 30 mgm. a day and later 20 mgm. a day over the next two months. The seven day period of administration remained the same. It is now the practice to start with 30 mgm. a day and reduce to 20 mgm. We find that unless the patient is very small the dosage cannot be decreased below 20 mgm. a day. The time of administration may also be reduced for most patients from a seven to a four day period. Oral progestogen has been quite as satisfactory in the relief of symptoms as true synthetic progesterone and its effect, as has been shown previously (15, 16, 17, 18) and can also be seen in Figure I, is physio-

logically the same. In the nine cases in which we have used pregneninolone there have been no untoward symptoms, either subjectively or objectively, and no masculinization has occurred.

REVIEW OF CASES

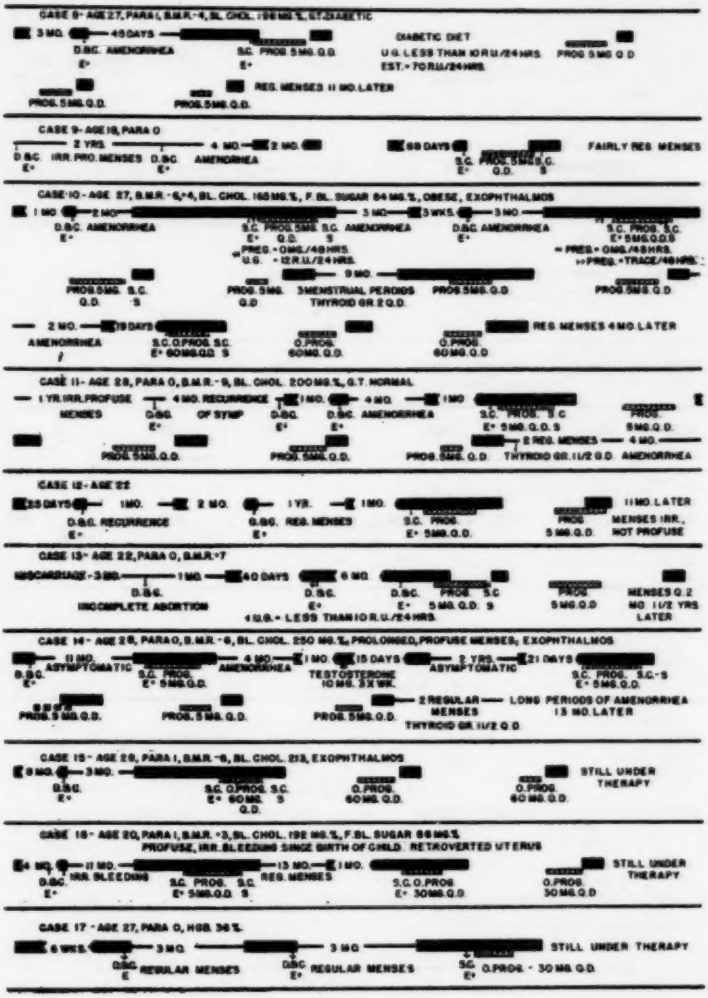
Four patients over 35 years of age were treated in order to evaluate the therapy in this age group. All responded satisfactorily, three following a single course of treatment (Cases 1, 2 and 3) and only one requiring more extensive therapy. This patient is still being observed (Case 4).

Fifteen cases fell in the age group below thirty-five. Of these, five (Cases 5, 6, 7, 8 and 9) have been apparently completely relieved of symptoms after one or more courses of progesterone and are now having regular menses. One of these (Case 7) is now pregnant. This patient had had four curettages performed over a period of six years and hyperplasia was found on each occasion. In the next two years she received two courses of progesterone therapy, obtaining relief on both occasions. Although this patient had a normal basal metabolic rate, her blood cholesterol was found to be somewhat elevated and therefore administration of thyroid extract was begun. Since the institution of thyroid therapy the patient's menses have been regular and she has had one miscarriage of a blighted ovum at three months. She is now four months pregnant again, this time with a normal hormone balance and an apparently normal fetus. Case 8, found to have early diabetes mellitus, was relieved of her menstrual difficulties by a single course of progesterone therapy and, with her diabetes controlled, is now having normal menses. Five patients (Cases 10, 11, 12, 13, and 14) are asymptomatic, having had either amenorrhea or irregular but not profuse menses over a period of from 2 years to 3 months after cessation of treatment. One of these (Case 10) has received four courses of progesterone over a period of as many years and has responded satisfactorily each time. Three cases (15, 16 and 17) are still under treatment, two with recurrences while the third is receiving therapy for the first time. In Case 18 there was associated chronic pelvic inflammatory disease and finally hysterectomy and bilateral salpingectomy were performed because of recurrent symptoms associated with this disease. Her bleeding

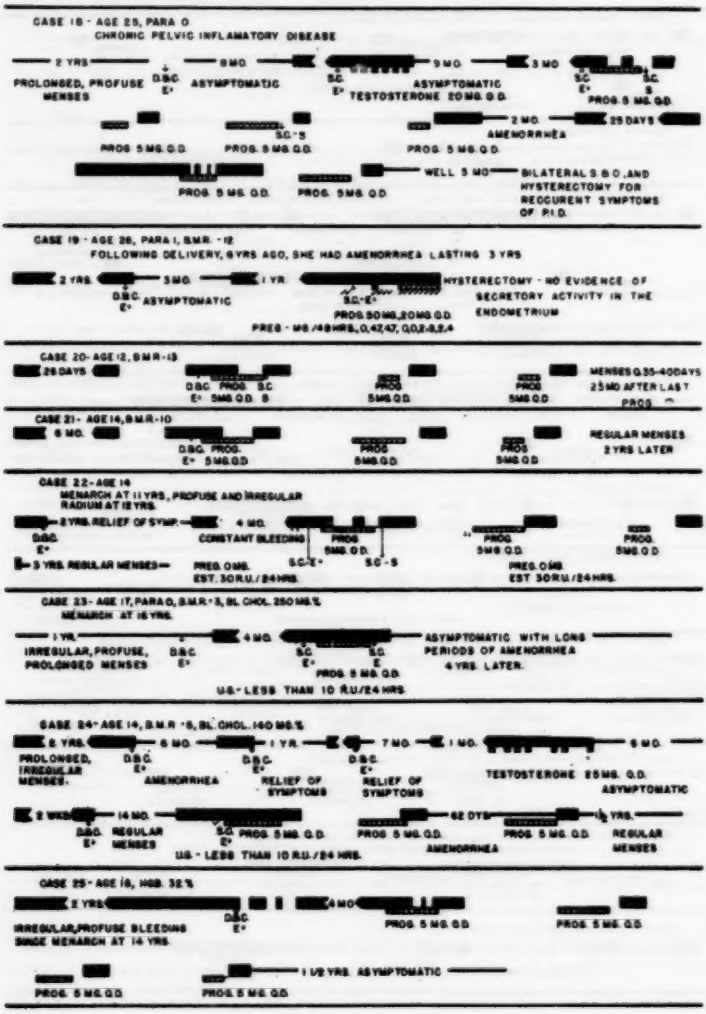


Case Histories

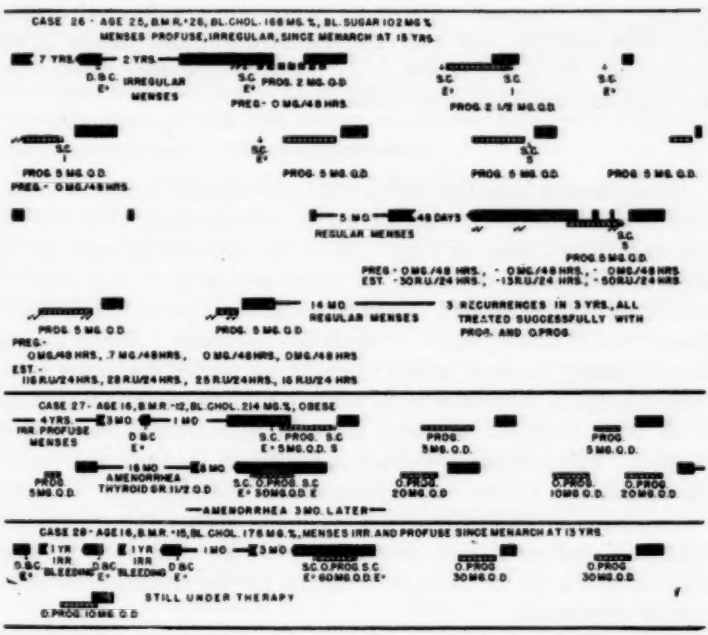
Legends: All bleeding phases are recorded as solid black areas with breaks to denote a lapse of time. Periods of treatment are marked by cross hatched areas, each cross denoting a day. Arrows represent curettages and checks endocrine determinations. S.C. denotes suction curettage; E+, endometrial hyperplasia; E, estrogenic endometrium; I, interval endometrium; S, secretory endometrium. Prog., refers to parenteral progesterone; O. Prog., Pregneninolone. Preg., denotes pregnanediol determinations; U.G., urinary gonadotropin; C.G., chorionic gonadotropin; Est., urinary estrogens.



CASE HISTORIES—*Continued*



CASE HISTORIES—*Continued*


CASE 26 - AGE 25, B.M.R. +26, BL. CHOL. 166 MG. %, BL. SUGAR 102 MG. %
MENSES PROFUSE, IRREGULAR, SINCE MENARCH AT 15 YRS.
7 YRS.
2 YRS.
IRREGULAR MENSES
PROG. 2 MG. Q.D.
PREG. - 0 MG./48 HRS.
PROG. 2 1/2 MG. Q.D.
PROG. 5 MG. Q.D.
PREG. - 0 MG./48 HRS.
PROG. 5 MG. Q.D.
PROG. 5 MG. Q.D.
PROG. 5 MG. Q.D.
5 MO.
48 DAYS
REGULAR MENSES
PROG. 5 MG. Q.D.
PREG. - 0 MG./48 HRS., - 0 MG./48 HRS., - 0 MG./48 HRS.
EST. - 50 R.U./24 HRS., - 13 R.U./24 HRS., - 50 R.U./24 HRS.
16 MO.
REGULAR MENSES
3 RECURRENCES IN 3 YRS., ALL TREATED SUCCESSFULLY WITH PROG. AND Q. PROG.
PROG. 5 MG. Q.D.
PROG. 5 MG. Q.D.
PREG. - 0 MG./48 HRS., .7 MG./48 HRS., 0 MG./48 HRS., 0 MG./48 HRS.
EST. - 116 R.U./24 HRS., 28 R.U./24 HRS., 25 R.U./24 HRS., 16 R.U./24 HRS.
CASE 27 - AGE 16, B.M.R. -12, BL. CHOL. 214 MG. %, OBESE
4 YRS.
3 MO.
1 MO.
IRR. PROFUSE MENSES
PROG. 5 MG. Q.D.
PROG. 5 MG. Q.D.
PROG. 5 MG. Q.D.
16 MO.
6 MO.
AMENORRHEA
THYROID GR. 1 1/2 Q.D.
PROG. 5 MG. Q.D.
PROG. 20 MG. Q.D.
PROG. 10 MG. Q.D.
PROG. 20 MG. Q.D.
AMENORRHEA 3 MO. LATER
CASE 28 - AGE 16, B.M.R. +15, BL. CHOL. 176 MG. %, MENSES IRR. AND PROFUSE SINCE MENARCH AT 13 YRS.
1 YR.
1 YR.
1 MO.
3 MO.
IRR. BLEEDING
IRR. BLEEDING
PROG. 30 MG. Q.D.
PROG. 30 MG. Q.D.
STILL UNDER THERAPY
PROG. 10 MG. Q.D.

CASE HISTORIES—*Concluded*

was satisfactorily controlled under progesterone therapy. Case 19 showed no response to progesterone therapy as judged by the microscopic appearance of the endometrium. This case, however, was one of the first treated and was not under our immediate care on the Johns Hopkins Hospital service.

Nine patients had had symptoms suggestive of endometrial hyperplasia since the onset of puberty. Only two of these (Cases 20 and 21) were treated with progesterone within the first few months after the onset of symptoms. Both patients are well, two and three years respectively, following a single course of progesterone therapy. One (Case 22), treated 4 years after the initial symptoms, has been well for a period of three years after a single course of progesterone. Three additional patients (Cases 23, 24 and 25) are asymptomatic following one series of progesterone treatments. Two patients (Cases 26 and

27) have had frequent recurrences necessitating additional therapy over three and five year intervals, respectively. One patient in this series (Case 28) is still receiving the initial progesterone therapy and her response has been quite satisfactory.

DISCUSSION

As can be seen from this study, of the twenty-eight patients treated with progesterone, twenty-six are well or asymptomatic as to bleeding and only in two cases or 7 per cent of the entire series were radical surgical procedures required. Of these two patients one was not treated on our service while the other had chronic pelvic inflammatory disease which finally made an operation necessary. Before progesterone therapy 14 cases, representing 22 per cent of the entire group under the age of thirty-five, or 37 per cent of those cases under the age of 35 with recurrent bleeding, required more radical methods of therapy. This is in sharp contrast to the 2 cases, or 7 per cent, requiring radical procedures under progesterone therapy. We feel that a comparison of these figures, although too small to be statistically significant, indicates that progesterone is an extremely valuable addition to the list of therapeutic agents for the treatment of endometrial hyperplasia.

The only serious objection to replacement progesterone therapy is a financial one. Unfortunately the cost of any form of progesterone is high.

If 5 mgm. of progesterone per day is given for ten days the first month of treatment, then for eight, six and four days the following months, the total cost of the drug for the entire four month course will be approximately fifty dollars. If further treatment is necessary four ampules of 5 mgm. each a month will usually suffice, making an additional cost of about eight dollars a month thereafter.

It oral progestogen is used, giving 30 mgm. in 10 mgm. tablets every day over a seven day period the first month and 20 mgm. a day for seven days during the next three months, the initial course of treatment will cost about twenty-eight dollars. If further therapy is necessary, 20 mgm. a day over a seven day period will usually suffice. This represents an additional cost of approximately six dollars a month. This is quite a financial advantage over the parenteral

method of administration in addition to the advantage of avoiding a daily office visit which is required by the parenteral method. Such prices are within the means of the average private patient, especially if by this treatment the cost and inconvenience of repeated hospitalization and operative procedures are saved.

If must be remembered that progesterone is only substitution therapy and every patient with endometrial hyperplasia should be thoroughly investigated for associated endocrine abnormalities. The basal metabolic rate, blood cholesterol and glucose tolerance tests are of especial value. As can be seen from a perusal of the appended case histories, a number of these patients are extremely obese. In this group reducing diets high in protein, low in carbohydrate and fat should be prescribed and the administration of thyroid extract may be tried even if the basal metabolic rate is within normal limits. Our results with thyroid therapy, however, as can be seen from an examination of the case histories, have on the whole been rather discouraging. Only one of our patients has apparently responded satisfactorily to thyroid medication.

Of seven cases in which the urinary gonadotropin titer has been determined, six were found to have less than ten rat units per twenty-four hours. The alcohol precipitation method (19) was used and the immature rat uterine weight served as the biological end-point. In one case approximately twelve rat units per twenty-four hours were found. Urinary estrogen assays have been performed in four cases. These showed as the highest values, for each individual patient, approximately sixty-three, seventy, eighty and one hundred and sixteen rat units of urinary estrogen for twenty-four hours. All of these determinations fall within the upper third of the range of values found in normal cyclic women in this laboratory. Repeated estrogen determinations in two patients showed variations within the normal range.

In five cases pregnanediol determinations were made on forty-eight hour specimens of urine before and after progesterone injections. The results of these studies have been reported earlier in more detail (13) but briefly stated no patient was found to excrete pregnanediol before therapy. Progesterone was recovered as pregnanediol following therapy in only two cases. From these results it was concluded that

progestational changes can be produced in the endometrium with doses of progesterone which are too small to be consistently recovered in the urine as pregnanediol.

SUMMARY

From a review of one hundred and eleven cases of functional uterine bleeding associated with the pathological picture of endometrial hyperplasia, it has been shown that 48 cases or 43 per cent of the patients are above the age of thirty-five years, or have passed the age when preservation of ovarian or childbearing functions is usually an extremely serious consideration. Of these, 22 patients or approximately half were treated successfully by a simple curettage. In one-quarter of the remaining cases there was some associated gynecological lesion necessitating surgical intervention. From these figures it has been concluded that the use of endocrine preparations in this age group is not imperative and surgery or irradiation therapy is the most practical method of treatment for the majority of cases. The choice between these two methods of treatment depends upon the presence of coexisting lesions, the preference of the patient or the proximity to the menopause.

Fifty-seven per cent of the cases, 63 patients, were below the age of thirty-five years. Again it seemed that there was no immediate indication for endocrine therapy as 40 per cent of these cases, 25 patients, required only a single dilatation and curettage for relief of symptoms. It was in the remaining 38 cases of this group in which it seemed that progesterone therapy might be of value, for 14 of these 38 patients or 37 per cent had required radical procedures for the relief of symptoms.

Of twenty-eight cases treated with progesterone only two required radical procedures, one because of failure of responses and the other because of associated chronic pelvic inflammatory disease. The reduction of the incidence of hysterectomy and irradiation therapy from 37 per cent to 7 per cent by the use of progesterone clearly indicates the value of the therapy.

For the successful use of progesterone in the treatment of endometrial hyperplasia the following facts must be considered:

1. Progesterone is substitution therapy and associated endocrine

abnormalities which may be contributing factors in the disease must be investigated.

2. The dosage and time interval of administration must be adequate.

3. Progesterone is not useful in checking immediate hemorrhage. It is used for preventive purposes and must generally be continued cyclically over a period of at least four months.

4. A recurrence of the disorder will not be prevented by progesterone.

Hormone studies performed on a selected group of cases showed no pregnanediol in the urine before treatment and no excessive urinary gonadotropin. Estrogen values were within the normal range.

The parenteral progesterone used in these studies was kindly furnished by Ciba Pharmaceutical Products, Inc, and Roche-Organon, Inc. The oral progestogen, Lutocylol, was furnished by Ciba Pharmaceutical Products, Inc.

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SPIROCHAETAL SURVIVAL IN FROZEN PLASMA

MARK M. RAVITCH AND JOHN W. CHAMBERS

From the Department of Surgery of the Johns Hopkins School of Medicine

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The rapid increase in the popularity of blood banks throughout the country and particularly the accumulation of large stores of plasma—liquid, frozen, or dried—have made important the study of survival of the *Treponema pallidum* in these various preparations. The possibility that under proper conditions, and particularly during the war, blood from syphilitic donors, treated or not, might be used for the preparation of plasma is undeniably worthy of serious consideration. The loss of possible donors and of blood already drawn and found to have a positive serological test for syphilis would be prevented, and the labor of making such determinations would be avoided.

Turner, Bauer, and Kluth (1) studied the viability of the spirochaetes of syphilis and yaws in desiccated blood serum. They found that under their experimental conditions the process of freezing and desiccation completely destroyed the infectivity of the spirochaetes. Turner and Diseker (2) found that *Treponema pallidum* inoculated in rabbit blood kept under conditions simulating those in the blood bank of the Johns Hopkins Hospital (3) failed to give rise to infection when injected into rabbits after storage periods of 48 hours or longer. Bloch (4), simultaneously performing almost parallel experiments, found some persistence of infectivity up to 72 hours but none at 96.

Since in a well run blood bank whole citrated blood should be used within two to three days after it has been drawn, there is no likelihood that any one will propose the use of syphilitic blood for whole blood transfusions. However, it may well be proposed that syphilitic blood be used for the preparation of plasma. Presumably, liquid plasma, being already more than a week old by the time the blood has been sedimented or been centrifuged and the plasma cultured and found sterile, would be safe. Desiccated plasma (1) would appear to be even safer.

The blood bank of the Johns Hopkins Hospital has been using simple

frozen plasma, not desiccated, since September 1941. Whenever possible, blood is centrifuged within 24 hours of drawing; the plasma is drawn off, pooled, redistributed, and frozen within the next 24 hours. No plasma is then released until cultures have been sterile for one week. There is thus a minimal period of nine days between bleeding and the earliest permissible use of plasma,—two days at ordinary

Treponema Pallidum in Human Plasma

DURATION OF STORAGE OF PLASMA AND SPIROCHETES	RABBIT NUMBER	INDURATION OF RIGHT TESTIS			SKIN LESIONS			DARK FIELD EXAMINATION		
		Pos.	Neg.	Days	Pos.	Neg.	Days	Pos.	Neg.	Days
0	1	+		37	+		20	+		37
0	2				Died 11 days					
0	3	+		35		−	35	+		35
24 hours	7	+		57		−	57	+		57
24 hours	8	+		86		−	95	+		95
24 hours	9		−		Died 22 days				−	
						−				
48 hours	13		−	94		−	94		−	94
48 hours	14		−		Died 75 days				Not done	
						−				
48 hours	15		−	94		−	94		−	94
72 hours	19		−		Died 84 days				Not done	
						−				
72 hours	20		−	96		−	96		−	96
72 hours	21		−		Died 76 days				−	
						−				
5 days	25		−	96		−	96		−	96
5 days	26		−	96		−	96		−	96
5 days	27				Died 19 days					
10 days	31		−		Died 47 days				−	
						−				
10 days	32		−		Died 60 days				Not done	
						−				
10 days	33		−	96		−	96		−	96

refrigerator temperature and seven in a freezing cabinet at −20 C. Freezing requires three to four hours. Thawing in a water bath at 37 C. requires 30 minutes.

On November 19, 1941, five rabbits were inoculated in each testis with 0.25 c.c. of the Nichols' strain of *Treponema pallidum*[1] just

[1] From the material maintained frozen at −78 C. in the Johns Hopkins School of Hygiene laboratory of Dr. T. B. Turner, whose help in this study is gratefully acknowledged.

previously thawed and found on dark field examination to contain about eight motile spirochaetes per field. On December 23, when most of the animals exhibited typical syphilitic orchitis, 42 gms. of trimmed testicular tissue from eight testes of these rabbits was ground with sand and 25 c.c. of beef infusion broth. The material was centrifuged five minutes and 20 c.c. of the supernatant fluid drawn off.

Treponema Pallidum in Rabbit Plasma

DURATION OF STORAGE OF PLASMA AND SPIROCHETES	RABBIT NUMBER	INDURATION OF RIGHT TESTIS			SKIN LESIONS			DARK FIELD EXAMINATION		
		Pos.	Neg.	Days	Pos.	Neg.	Days	Pos.	Neg.	Days
0	4	+		26	+		45	+		30
0	5	+		30	+		30	+		30
0	6	+		26	+		26	+		30
24 hours	10	+		79		−	79	+		79
24 hours	11		−		Died 69 days			Not done		
						−				
24 hours	12		−	95		−	95		−	95
48 hours	16		−		Died 80 days					
						−			−	
48 hours	17		−	94		−	94		−	94
48 hours	18		−		Died 75 days			Not done		
						−				
72 hours	22		−		Died 74 days			Not done		
						−				
72 hours	23		−	97		−	97		−	97
72 hours	24		−		Died 55 days				−	
5 days	28		−	96		−	96		−	66
5 days	29		−	96		−	96		−	96
5 days	30		−		Died 41 days				−	
						−				
10 days	34		−	96		−	96		−	96
10 days	35		−	96		−	96		−	96
10 days	36		−	90		−	90		−	90

Dark field examination showed eight to ten actively motile spirochaetes per field. Of this material 10 c.c. was added to 90 c.c. of stored, pooled human plasma which had been frozen and thawed, and the other 10 c.c. added to 90 c.c. of rabbit plasma five days old (refrigerated at 5 C.).

Each of the 100 c.c. lots of inoculated plasma was divided into six batches of 16 c.c. each. Rabbits 1, 2, and 3 were inoculated at once with the human plasma, and rabbits 4, 5, and 6 with the rabbit plasma,

—0.25 c.c. in the right testis and 0.1 c.c. in five points intradermally. All the remaining tubes of inoculated plasma were placed in the freezing cabinet at −20 C. At intervals of 24, 48, 72 hours, 5 days, and 10 days succeeding pairs of tubes were thawed and the plasma was inoculated into rabbits, three with human plasma, three with rabbit plasma. These animals all received twice the volume of material injected into the controls; that is to say, 0.5 c.c. in the right testis and 0.2 c.c. intradermally at five points. Thawing at 37 C. in the water bath required five minutes and injections were usually completed within an hour.

The table shows that one of the six controls died after 10 days and that in all the others there developed lesions from which motile Treponemata could be recovered. In the 24 hour group three out of five were infected, the incubation periods were much longer, and no skin lesions developed.

No animals in the succeeding groups showed lesions. The right testis of those which died of intercurrent infections was in almost every instance triturated with sand and broth, and the material was centrifuged and examined under the dark field microscope. Treponemata were never found. Those rabbits which survived three months were sacrificed and similarly examined. Treponemata were not recovered from any of them. It is worthy of note that two of the controls died of intercurrent infections and that their testes when examined by this technique showed good numbers of characteristic motile spirochaetes.

We are aware that lymph node transfers might have been done to prove beyond doubt the absence of asymptomatic syphilis in the animals which failed to become infected. The results were so uniform that such transfers seemed unnecessary.

It is interesting, of course, that our source material was also frozen. The organisms tolerate the greater cold of −78 C. for as long as three years. Turner and Brayton (5) have shown that storage temperature is more important than freezing temperature or rate of freezing. Rapid thawing—as in these experiments—favors spirochaetal survival.

SUMMARY

Human and rabbit plasma heavily inoculated with *Treponema pallidum* and frozen at −20 C. for 48 hours or longer is not infectious

for normal rabbits. The material is infectious, however, when the freezing period is only 24 hours. Under these conditions the incubation period is prolonged even though the experimental animals receive an inoculation dose twice the size of that given the controls.

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A SULFONAMIDE FILM FOR USE AS A SURGICAL DRESSING

Preliminary Report

KENNETH L. PICKRELL, M.D.

Department of Surgery, The Johns Hopkins University and Hospital

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In view of the great effectiveness of the newer chemotherapeutic agents in combating infection, it was thought that these might be used advantageously in patients with burns and various other infected lesions. An aqueous sulfadiazine solution in triethanolamine was introduced (1), which has been found to be effective in the treatment of burns, in combating infection in the paranasal sinuses and reducing the symptoms and complications associated with the common cold (2), for irrigating infected wounds and sinuses, in preparing the surgical site for operations in or around the body orifices, eyes, orbits, and so forth, and as a medium to combat infection before and after skin grafting.

However, this solution, when used on burned patients, had several disadvantages. Since it was an aqueous solution, the drying time was slow as compared with other forms of treatment, and the film formed over the burned surface was thin and fragile. In an attempt to remedy these difficulties, methyl cellulose[1] has been added, and this has definitely enhanced the drying and film forming properties of the solution. While studying various drying agents and plastic substances, it occurred to the writer that a preformed sulfonamide film might serve a very useful purpose in surgery. About one year ago we made our first sulfonamide films which were rather crude. Our technic has been altered somewhat, so that the resulting film is thin, strong, pliable, slightly elastic, and translucent.

The films are made in the following manner: An emulsion is prepared containing 3 per cent sulfadiazine or 3 per cent sulfanilamide, 2.5 per cent methyl cellulose,[1] 3 per cent triethanolamine and 0.5 per cent sorbitol, with 50 per cent alcohol or acetone added to make 100 cc. The resulting emulsion is sprayed on a smooth, horizontal glass surface

[1] Methyl cellulose—Dow Methocel (15 centipoise).

with a pressure gun or paint spray apparatus, and allowed to dry. When acetone is used as the drying agent evaporation is very rapid, but when 50 per cent alcohol is used, several hours at 75°C. are required for drying before the film can be easily removed in a single sheet. The sheets can be made in any desired size. The films are stable and can be sterilized by dry heat. Since the film retains only a slight amount of moisture, composition studies indicate that it ordinarily contains 35 to 50 per cent sulfonamide. When a segment of the film is placed beneath the skin of a rabbit, absorption takes place, and the sulfonamide can be detected in the blood within several hours. At the end of twenty-four hours, disintegration of the film is almost complete.

We have used these prepared sulfonamide films in more than 100 cases; about fifty have been patients with burns. In these cases a surgical deturgent is used to clean the burned surface and the surrounding skin if the area is grossly contaminated. The area is then washed with saline, sulfadiazine, or azochloramid solution. While the surface is still moist the sulfonamide film is placed on the burned surface, a good margin being allowed, since the size of a burn after twenty-four or forty-eight hours may greatly exceed its original visible size. Although the film is as pliable as thin paper, trimming may be necessary in areas such as the axilla or web spaces of the hands. A smooth, firm pressure-dressing of gauze is then applied. It has been our practice to allow this film to remain in place from three to five days, at the end of which time, in second degree burns, epithelization will be taking place. Since the film is translucent, the injured area may be inspected without removing it. In third degree burns, and in exudative lesions, the film may be renewed as desired.

These films have been used in more than fifty additional instances: to cover recent incisions, lacerations, and abrasions; to prepare granulating areas for grafting; to cover the recipient and donor areas at the time of grafting; to cover abraded and ulcerated areas; and as a framework to hasten closure and regeneration of perforated ear drums.

Although bacteriologic studies have not been carried out in each instance with the different sulfonamide concentrations in the various films, in 30 instances in which films of the above mentioned sulfonamide content have been used for burns, and in which bacteriologic studies

have been made, no infection has been encountered. In the remaining cases, there has been no evidence of infection on inspection of the wounds. When low concentrations of the sulfonamides have been used in the films, infection has occurred. It is our impression that the sulfadiazine films offer a greater measure of local protection against infection than do the sulfanilamide films.

We feel that preformed sulfonamide films of this character may find a definite place in surgery, in the hospitalized and ambulatory patient, and in cases in which infection is imminent, where surface drying is desired, or where moisture, coincident with the use of ointments and resulting maceration, is to be discouraged.

The author is indebted to Dr. Anthony M. Moos of the Lederle Laboratories for his assistance and cooperation in preparing and analyzing the films.

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BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal.)

Time and the Physician. The autobiography of LEWELLYS F. BARKER. 350 pp. Illus. $3.50. *G. P. Putnam's Sons, New York,* 1942.

Still another story of a busy and productive life has been added to others dealing with eminent physicians who were important in the early years of the Johns Hopkins Medical School and Hospital. While autobiographical, this book retains an admirable detachment: the writer seems to see himself as part of certain trends, social, scientific, pedagogic and philosophic. Thus, Dr. Barker becomes historian for present purposes and gives us an excellent account of life in Canada in the latter part of the last century, his experiences with Mall, then his brief career at the University of Chicago, and, finally, his important connection with the medical life of Baltimore. Most interesting to the majority of local readers will be his story of the early days of the Medical School and Hospital, and of the shift of the clinical departments to the University basis, which interrupted his incumbency as Professor of Medicine.

The author disclaims ability to express his emotions; nevertheless, his remarks about certain colleagues (especially, perhaps, Thayer) are more warmly appreciative than would be required by mere courtesy. Speaking of the "College of American Physicians and Surgeons", "founded in 1887", the author doubtless referred to the "Congress of American Physicians and Surgeons". With this minor exception, the book is excellent history of an era, in so far as the limits of this reviewer's knowledge permit him to judge.

J. T. K.

Principles and Practice of Cardiology. By CRIGHTON BRAMWELL and JOHN T. KING. 509 pp. Illus. $12.00. *Oxford University Press, London: Humphrey Milford,* 1942.

This textbook of clinical cardiology represents a collaboration by a British and an American author which may be symbolic and appears to have been successful.

The authors have taken a leaf from textbooks of pathology in organizing the book into two sections, General and Special Cardiology. After that the association with pathology ceases, since the discussion of anatomical changes per se has been minimized or omitted entirely wherever possible. The major emphasis has been placed on the clinical, physiological and etiological aspects of heart disease.

In Part I Doctor Bramwell describes lucidly the basic principles involved in obtaining and interpreting the subjective and objective evidences of cardiac disease at the bedside. These are supported by discussions of pertinent physiological data, citation of cases and reproductions of diagrams and X-rays. There are also chapters on the interpretation of electrocardiographic tracings, on heart disease in pregnancy and finally on general principles of therapy and prognosis.

In Part II Doctor King takes up each etiological type of heart disease and describes the cause, incidence, clinical findings, prognosis and treatment in a concise and complete manner. The discussions of modern treatment are broad enough to include not only the author's preference, but also other methods in general use.

Students will undoubtedly appreciate the simplicity and clarity of style of the authors and may even conclude that to learn cardiology is not a difficult task after all.

L. K. A.

Psychotherapy in Medical Practice. By MAURICE LEVINE. 320 pp. $3.50. *Macmillan Co., New York*, 1942.

The author is a former Hopkins internist and psychiatrist who has had excellent psychoanalytic training. His goal is to present a practical, clear-cut statement of the methods of psychologic treatment to the general practitioner and medical student. The first chapter deals with such common misconceptions as the statement that heredity is the chief cause of psychiatric disorders, that masturbation causes psychoses or physical disease, that overwork is the cause of mental disease, that there is a sharp difference between "normal" and "abnormal," and that sex experience or marriage cures all psychiatric disorders. Twenty-four misconceptions are listed and briefly discussed.

The next three chapters present the methods of psychotherapy best suited to the general practitioner. Twenty-five "techniques or elements of psychotherapy" are described, but it is emphasized that they are "not sharply defined, but shade over into each other." There are included the therapeutic value of such reassuring routine procedures as the physical examination and physical treatment, as well as practical suggestions for the use of occupational therapy, hobbies and the establishment of a daily routine. Such inter-personal activities as authoritative firmness, guidance and advice, provision for fears and inferiority feelings and the satisfaction of frustrated and neurotic needs are described, to mention but a part of the armamentarium listed by the author.

The topics are discussed in a matter of fact manner; too technical or controversial issues are avoided. All of the concepts are presented in a simplified form, but they are not over-simplified or mechanized. Detailed case studies are omitted but clinical examples, in keeping with the needs of everyday practice, are frequently introduced and aptly stated. There are adequate chapters dealing with suicide risks, sex and marriage and basic attitudes toward children. One of the best written chapters deals with the concept of normality and maturity. An excellent bibliography and index are included.

Although the ideal psychiatric textbook for the general practitioner has yet to be written, Dr. Levine's book comes closest to it by providing a balanced, common-sense, clearly written account of what the general medical man can accomplish by psychotherapy.

J. H. C.

Personality and Mental Illness, An Essay in Psychiatric Diagnosis. By JOHN BOWLBY, M.D. 280 pp. $2.75. *Emerson Books, Inc., New York,* 1942.

The author is a British psychiatrist who became fascinated by the question, What sort of man was the patient before he became ill? Gradually he "came to recognize certain characteristic personalities who seemed specially liable to develop a functional mental illness." Sixty-five case records are presented and analyzed into specific and non-specific character traits. The author concludes that 33 traits have specific significance for diagnostic purposes. He emphasizes that no trait could be found which was common among affected individuals with manic-depressive conditions although certain traits did appear to be peculiar to schizoids. But even such "specific" schizoid traits, as "solitary," "no humor," "saint like," "apathetic," "occult interests," were not found to be absolutely specific. Since traits are dispositions to act in certain ways in specific situations and vary from case to case as well as from time to time, it would be surprising if the author had arrived at any other conclusion.

For the purpose of determining diagnosis and prognosis it is suggested that there be established in each case: (1) the genetic type (syntonic; schizoid); (2) the personality sub-type (cautious obsessive; saint like; hysterical; etc.) (3) the degree of instability (very stable to very unstable); (4) the present state and its setting. This outline is incomplete and lifted out of its context but it indicates that the author's diagnostic-prognostic procedure provides no facile categories into which personality may be conveniently pigeon-holed.

The book will be of interest to physicians as well as to psychiatrists as it includes a review of the methods utilized in measuring personality. An adequate bibliography and index are included.

J. H. C.

Morris' Human Anatomy. Edited by J. PARSONS SCHAEFFER. 10th ed. 1635 pp. Illus. $12.00. *The Blakiston Company, Philadelphia,* 1942.

The tenth edition of this standard text-book of human anatomy, the first in nine years, appears under a new editor. There also are new authors responsible for the sections on "Skin and Mammary Glands", "Musculature", "Cardiovascular System", "Nervous System", and "Digestive System", which have been considerably rewritten and rearranged. Many new figures have been added or replace old ones. Bibliographies have been revised and largely brought up to date. The BNA terminology is used throughout; but the value of including INA synonyms especially when excluding those of the BR may be questioned.

It is gratifying to note that Bardeen's arrangement of the muscular system has been retained. In the opinion of the reviewer, this arrangement, while not ideal, is the best found in any standard text-book written in the English language.

W. L. S., JR.

History of the School of Nursing of the Presbyterian Hospital, New York, 1892–1942. By ELEANOR LEE. 286 pp. Illus. $3.50. *G. P. Putnam's Sons, New York, 1942.*

Wherever we live at the present time history is playing an important rôle in the trend of events for 'the past is the present and the present is the future.' The nursing profession has a history which is far from being static and it has constantly met the challenge of adjusting to new conditions as they arise.

The year 1942 marks the occasion of the fiftieth anniversary of the founding of the School of Nursing at the Presbyterian Hospital in New York and Eleanor Lee has gathered together all the data relative to the establishment and progress of this notable institution. The material presented by Miss Lee, with such knowledge and skill, represents not merely a collection of facts, but also a story of the events that have occurred year by year in the development and growth of the nursing school. It is in the nature of a biography as well as a history, for the author dwells upon the lives of some of the nurses—graduates of the school who have made outstanding contributions to the historical development of the school as well as to the nursing profession in general.

As a whole, this important volume professes to be a complete saga, with frequent pictorial descriptions of everything that has occurred to build and maintain the reputation of this fine school. In the beginning of the book the author describes conditions as they were in New York during the latter half of the nineteenth century, when the care of the sick in hospitals left much to be desired. There were already established in the city of New York six hospitals, but this was found to be inadequate. An appeal was therefore made, in all Presbyterian Churches, for funds to build another hospital to take care of the poor and destitute. The response was so encouraging that in October, 1872, the hospital was formally opened as "The Presbyterian Hospital for the Poor of New York, without regard to Race, Creed or Color."

During the next twenty years the hospital continued its splendid work of caring for the sick and new buildings were added. In 1892 Anna C. Maxwell, a graduate nurse of the Boston City Hospital, was appointed Superintendent of Nurses, and, largely through her efforts the Training School of the Presbyterian Hospital was organized and established. Good judgment and sound common sense, together with a practical idealism regarding nursing in general, characterized Miss Maxwell's work and have left an indelible impression upon the school which she founded and to which she gave loyal service for a period of thirty years.

In 1921 Helen Young, a graduate of the school and later assistant to Miss Maxwell, was appointed her successor. From then onward many changes have taken place in the school. Noteworthy among these was the agreement between Presbyterian Hospital and Columbia University to establish a University Course in Nursing from which the students are graduated with a Bachelor of Science degree in nursing.

The School of Nursing at the Presbyterian Hospital has attracted young women of the finest calibre and social standing. Under the influence and guidance of Miss Maxwell and Miss Young two thousand of these alumnae have graduated, many to accept positions of considerable import in the nursing world; others to join the armed forces in the service of their country. The past fifty years have

been fruitful years in the history of this school. The next period will be built on this excellent foundation.

J. B. B.

Communicable Disease Nursing. By THERESA I. LYNCH. 678 pp. Illustrated. $3.75. The C. V. Mosby Company, St. Louis, 1942.

Miss Lynch, who was formerly in charge of the Willard Parker Hospital for Communicable Diseases, is well qualified to write a text on communicable disease nursing because of her wide experience in this field.

A short historical introduction precedes the chapters on prevention and control of this type of disease, on vaccines, immune sera and serum reactions. Medical aseptic technic is discussed fully and is generously illustrated with series of photographs explaining the steps in carrying out the technic in a communicable disease unit as well as in the wards of a general hospital and in the home. Full explanation is given of the method of carrying out treatments, the care of equipment, and the disinfection of contaminated materials.

The chapters on the various diseases follow the same general outline and have bold-faced type paragraph headings to facilitate the use of the volume for quick reference. The headings include a short history, the prevalence, source of infection, mode of transmission, susceptibility, pathology, incubation period, course of the disease, complications and sequelae, points of differential diagnosis, period of communicability, prognosis and mortality, treatment, methods of control, quarantine, and immunization. The discussion of nursing care includes, besides the important points in the care of the patient, isolation precautions and disinfection, and community protection.

A list of references and suggested reading follows the discussion of each disease. The volume is generously illustrated and includes a number of photographs in color as well as charts and clinical records. The public health aspects are stressed throughout. The chapter on home care of communicable diseases is excellent; many practical suggestions and illustrations are given to assist in setting up isolation precautions with equipment found in the average home.

Although this book is written primarily as a text for nurses, there are many explanations and practical suggestions which should be of interest to practicing physicians, especially to those who come in contact with communicable diseases in the home.

M. S.

Ornithologists of the United States Army Medical Corps. By EDGAR ERSKINE HUME. 583 pp. Illus. $5.00. The Johns Hopkins Press, Baltimore, 1942.

Colonel Hume has gathered the biographical data of 35 officers of the Medical Corps of the U. S. Army who have contributed directly or indirectly to the store of bird lore. Some like Leonard Wood and Sternberg are included because of the support they furnished to officers interested in natural history. Others like Coues, Hammond and the fantastic Xántus were eminent authorities in zoology and their names are remembered in the official nomenclature of many birds and mammals.

Many a young medical officer assigned to a frontier post before the turn of the century was encouraged by Baird of the Smithsonian Institute to become an individual field expedition; the zoological exploration of the West was carried out in large part by the men whose activities have been recorded here. Colonel Hume has given us a series of scholarly vignettes which should prove most interesting for ornithologists but, in addition, there is many an absorbing sketch of Indian War days for less specialized readers. The volume is profusely illustrated and faultlessly published.

J. L. L., Jr.

BOOKS RECEIVED FOR REVIEW

Physical Chemistry for Students of Biochemistry and Medicine. By Edward Staunton West. 368 pp. Diagrams. $5.75. The Macmillan Company, New York, 1942.

Report of Medical Advisory Committee on the Use of Mass Miniature Radiography in the Detection of Pulmonary Tuberculosis among Recruits for H. M. Forces. Cmd. 6353. 6 pp. Five cents. His Majesty's Stationery Office, London, 1942.

How to Live in the Tropics. By Virginia Lloyd Hunt. 178 pp. $2.00. Harcourt, Brace and Company, New York, 1942.

Edinburgh Post-Graduate Lectures in Medicine. Volume 2. 540 pp. Illus. 12/6. Published for the Honyman Gillespie Trust by Oliver and Boyd, Edinburgh and London, 1942.

The Reception of William Beaumont's Discovery in Europe. By George Rosen. 97 pp. $5.00. Schuman's, New York, 1942.

Textbook of Gynecology. By Arthur Hale Curtis. 4th ed. 723 pp. Illus. $8.00. W. B. Saunders Company, Philadelphia and London, 1942.

Pleuro-subpleural Zone. By J. Skládal. 103 pp. Illus. $2.75. Cambridge: at the University Press; New York: The Macmillan Company, 1942.

Gynecology and Female Endocrinology. By Emil Novak. 605 pp. Illus. $10.00. Little, Brown and Company, Boston, 1941.

Synopsis of Pathology. By W. A. D. Anderson. 661 pp. Illus. $6.00. The C. V. Mosby Company, St. Louis, 1942.

Professional Adjustments II. By Gene Harrison. 424 pp. $3.50. The C. V. Mosby Company, St. Louis, 1942.

The Hand—Its Disabilities and Diseases. By Condict W. Cutler. 572 pp. Illus. $7.50. W. B. Saunders Company, Philadelphia and London, 1942.

Bibliography of Aviation Medicine. By Ebbe Curtis Hoff and John Farquhar Fulton. Prepared for the Committee on Aviation Medicine, Division of Medical Sciences, National Research Council, acting for the Committee on Medical Research, Office of Scientific Research and Development, Washington, D. C. 237 pp. $4.00. Charles C. Thomas, Springfield, Illinois, 1942.

An index very useful for those working in this field.

War Medicine. A Symposium. Edited by WINFIELD SCOTT PUGH [and others]. 565 pp. Illus. $7.50. Philosophical Library, New York, 1942.

Biological Action of the Vitamins. A Symposium. Edited by E. A. EVANS, JR. 227 pp. Illus. $3.00. University of Chicago Press, Chicago, 1942.

Advances in Internal Medicine. Vol. 1. Edited by J. MURRAY STEELE [and others]. 292 pp. Illus. $4.50. Interscience Publishers, New York, 1942.

Advances in Pediatrics. Vol. 1. Edited by ADOLPH G. DESANCTIS. 306 pp. Illus. $4.50. Interscience Publishers, New York, 1942.

Human Pathology. By HOWARD T. KARSNER. 6th ed. 817 pp. Illus. $10.00. J. B. Lippincott Company, Philadelphia, 1942.

TOPICAL PROJECTION OF NERVE FIBERS FROM LOCAL REGIONS OF THE COCHLEA TO THE CEREBRAL CORTEX OF THE CAT[1]

CLINTON N. WOOLSEY AND EDWARD M. WALZL

Department of Physiology and the Otological Research Laboratory, The Johns Hopkins University, School of Medicine, Baltimore, Md.

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The purpose of these experiments was to determine directly by physiological methods the manner in which cochlear nerve fibers in the osseous spiral lamina project to the auditory area of the cerebral cortex.

At the time the investigation was initiated (June, 1940), considerable experimental evidence indicated that the cochlea undoubtedly has some sort of "point-to-point" representation in the cortex. This seemed clear from a consideration of (1) the anatomical studies of Lorente de Nó (1933a) and of Lewy and Kobrak (1936) on the cochlear nucleus; (2) the physiological observations of Ades, Mettler and Culler (1939) and of Coakley and Culler (1940) on the medial geniculate body of the cat; and (3) the anatomical investigations of Poliak (1932) on the auditory radiation and of Walker (1938) on the relations of the cerebral cortex to the medial geniculate body of the monkey. However, the precise, and indeed the general, arrangement of the cortical projection in terms of peripheral structures remained to be established. Attempts to demonstrate localization of electrical response in specific parts of the cortex on stimulation of the ear with pure tones (Davis, 1934; Bremer, 1939) had not succeeded in revealing the existence of localization within the total cortical area responsive to click stimulation (Kornmüller, 1933, 1937; Davis, 1934; Bremer, 1939; Bremer and Dow, 1939; Ades, 1941a). In fact, the only physi-

[1] A preliminary account of this work was given before the American Physiological Society in Chicago on April 18, 1941. Initial reports on two other studies dealing with the same general subject, but employing different technics, were made at approximately the same time. Ades' (1941b) paper was presented a week earlier at the meeting of the American Association of Anatomists; Licklider (1941) gave his before the Eastern Psychological Association (April 18–19, 1941).

ological evidence for tonal localization at the cortical level was that of Girden (1940) who had found transitory impairments of acuity for different frequencies in two dogs studied with conditioning and ablation technics. Later Lipman (1940) made a preliminary report on a similar study. In the clinical literature evidence for tonal localization in the auditory cortex had been offered by Pfeifer (1936) and others.

Since the fibers of the cochlear nerve are distributed in an orderly manner to the organ of Corti (Lorente de Nó, 1937), and since localized regions of the latter respond optimally to specific frequencies of the sound spectrum (Crowe, Guild and Polvogt, 1934; Stevens, Davis and Lurie, 1935; Culler, 1935; Walzl and Bordley, 1942), it seemed that demonstration of a "point-to-point" projection of the fibers of the cochlear spiral to the cerebral cortex should provide an anatomical basis for tonal localization in the cerebral mantle. By stimulating electrically small groups of nerve fibers in the osseous spiral lamina and by defining areas of cortex in which such stimulation evoked electrical potentials, we have been able to show that fibers from specific regions of the cochlea project to corresponding, specific portions of the auditory cortex.

MATERIAL AND METHODS

Fourteen young adult cats were used for focal stimulation of the cochlear nerve. In each animal small groups of nerve fibers were stimulated electrically at one or more points along the free edge of the osseous spiral lamina. In the whole group 31 points were thus excited. Of these 19 were in the basal, 3 in the middle and 9 in the apical turn. In addition, the entire eighth nerve was stimulated in 4 animals.

Each animal was deeply anesthetized with phenobarbital- or pentobarbital-sodium, administered intraperitoneally. Throughout the experiment the anesthesia was kept at a level sufficient to reduce the spontaneous electrical activity of the cortex to a minimum.

Dissection of the cochlea was made through the tympanic bulla. After tracheotomy, the bulla was exposed by separating the structures ventral to it, and the middle ear was entered by cutting away the bulla and removing the septum. Under a binocular dissecting microscope the bony capsule of the cochlea was removed with the aid of a fine dental burr and a small chisel. Thus the basal, middle and apical turns of the osseous spiral lamina came into view as shown in the photograph of Fig. 1. In removing the bony capsule the organ of Corti was separated from the nerve fibers along the edge of the spiral. In 11 experiments the *left* cochlea, in 2 the *right* and in one both cochleae were dissected.

In all animals the *right* cortex was exposed widely to permit systematic examina-

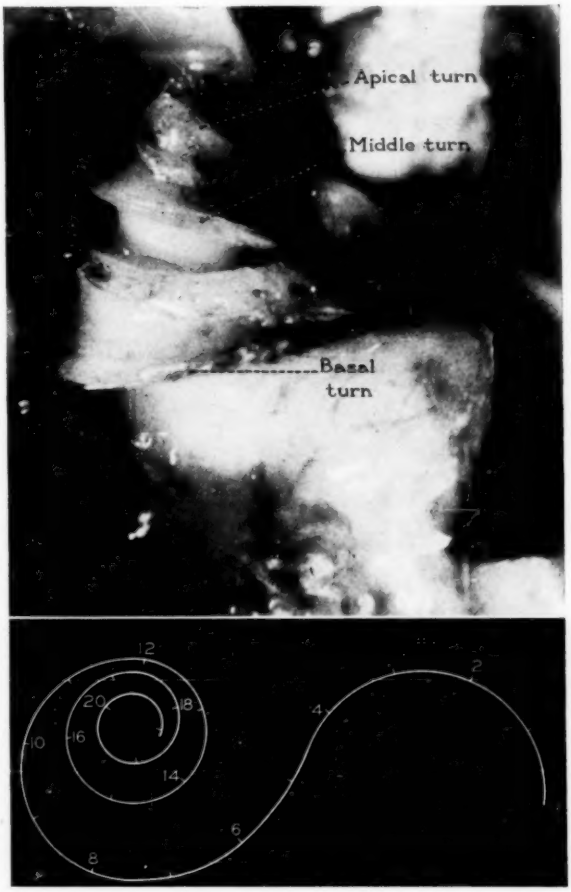

FIG. 1. Photograph, 15×, showing ventral aspect of basal, middle and apical turns of the osseous spiral lamina of the left cochlea of the cat, exposed to permit application of fine wire, stimulating electrodes to the free edge of the lamina. The diagram of the spiral below the photograph is on approximately the same scale (12×). It is marked off in 1.0 mm. gradations from 0.0 mm. at the basal end to 21.0 mm. at the apex. The region 9 mm. to 13 mm. inclusive, lying on the cranial aspect of the cochlea, was inaccessible to stimulation. Dissection by E. M. W.

tion of the lateral surface with the exploring electrode. The animal's head was fixed firmly in a holder with the lateral aspect of the exposed hemisphere uppermost. A stereotaxic instrument was placed in position so that the exploring electrode (made of steel tubing and a core of No. 50 cotton thread moistened with Ringer's solution)could be moved over the surface of the cortex in millimeter steps alon g rectangular coordinates. The reference electrode usually was placed on the skull at a distance from the auditory areas of both hemispheres. To prevent drying, the exposed surface of the cortex was flooded with mineral oil. This procedure also restricted the area of contact between the moist thread electrode and the pia-arachnoid and gave more constant recording conditions than without the oil.

Electrodes for stimulation of the cochlear nerve fibers were made from fine, No. 40, stainless steel wires shellacked to the tips. One or more pairs of these electrodes were placed with their tips in the groove of the free edge of the osseous spiral lamina. This was done with micromanipulators under a low-powered binocular microscope. The tips of each pair of electrodes were approximately 0.7 mm. apart. The average number of nerve fibers per millimeter of spiral is probably about 2500.[2] How many were stimulated at one time we do not know, but reversing the direction of current flow through the electrodes regularly produced a shift in the locus of maximal response at the cortex, suggesting that stimulation was rather well limited to fibers in the region of the cathode. The evidence indicates that stimulation was less well restricted at the apical end of the cochlea than at the base, as might be expected from the structure of the cochlea.

Stimuli employed were condenser discharges from a thyratron stimulator delivered to the electrodes through an isolation transformer. The strength of stimulus varied from one experiment to another but effort was made to use the weakest stimulus adequate to produce a good response. Generally this was not much above threshold for the minimal response. Stimuli were applied in short series at a rate of approximately one per second. At this interval the effect of the preceding stimulus had disappeared. There was no difficulty in maintaining the excitability of a group of fibers during the course of an experiment, and it was not necessary to increase the strength of stimulus periodically.

The cortical responses were amplified with a three-stage, resistance-capacity coupled amplifier (Johnson Foundation Model No. 39), utilizing 0.1 or 1.0 sec. coupling condensers. Responses were visualized on a 3 inch RCA cathode ray oscilloscope (Model No. 155), modified to permit single sweep operation in synchrony with the stimulus (Talbot, 1941). Records were made by photographing the cathode ray trace on supersensitive 35 mm. film with a Leica camera.

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[2] The average number of cells in the spiral ganglion of the cat is about 50,000 (Howe, 1935). The average length of the cat's cochlea, as measured at the junction of the inner and outer pillar cells, is 21.5 mm. (Walzl and Bordley, 1942). Possibly in cat as in man the number of cells per mm. differs in various parts of the cochlea (Guild, Crowe, Bunch and Polvogt, 1931).

Protocols were kept of the cortical points examined and of the photographs taken. Each point was numbered and its position marked on coordinate paper. The positions of sulci also were noted. Subsequently photographs of the responses were mounted on an enlarged drawing of the brain in positions corresponding to the points at which the records were taken. The scale was such that each record occupied an area on the chart equal to one square millimeter of cortical surface. The brain drawings in each case were made from photographs of the actual brains studied.

RESULTS

A. The pattern of localization in the primary auditory area of the cerebral cortex

On stimulating electrically small groups of nerve fibers in the spiral osseous lamina, it was found that the cortical area of response in each case was limited to a portion of auditory area and varied in position within the total area according to the part of the cochlea stimulated. This made it possible to dissect functionally the cortical system in terms of the site of origin of the cochlear nerve fibers and to define in a "point-to-area" fashion the relations of the cochlea to the cerebral cortex. Although the cochlea is represented bilaterally in the cortex, we shall first describe the relationships as they exist in one hemisphere.

Fig. 2 shows the surface positive electrical potentials[3] recorded from the right cortex in Expt. 1/12/41 when the cochlea was stimulated focally at a point 5 mm. from the basal end of the spiral. The area of response is situated just caudal to the upper end of the anterior ectosylvian sulcus and extends dorso-ventrally in a band-like manner through this portion of the auditory area. It is characterized by a *focal area* in which the responses are large (approx. 300 μV) and by a fringe in which the size of responses progressively diminishes to zero. In general the change in amplitude is more rapid rostro-caudally than dorso-ventrally. The dots mark points examined at which no responses occurred.

In Expt. 3/5/41 (Fig. 3 and Fig. 9a) fibers were stimulated first at

[3] Under deep nembutal anesthesia the cortical response to nerve stimulation is almost purely surface positive. With lighter anesthesia and on click stimulation of the intact ear the form of the response is more complicated and may show negative phases preceding and following the positive wave (see Hawkins, 1941). In this paper we are considering only the surface positive response.

the basal and then at the apical end of the contralateral cochlea. The cortical area related to the 0.5 mm. point lies above the dorsal end of the anterior ectosylvian sulcus, and that related to the 19 mm. point is situated in the posterior ectosylvian gyrus. The experiment demonstrates a clear separation of the cortical areas for the basal and apical ends of the cochlea and defines the positions of these areas within the auditory area. It also shows that the auditory area may extend well

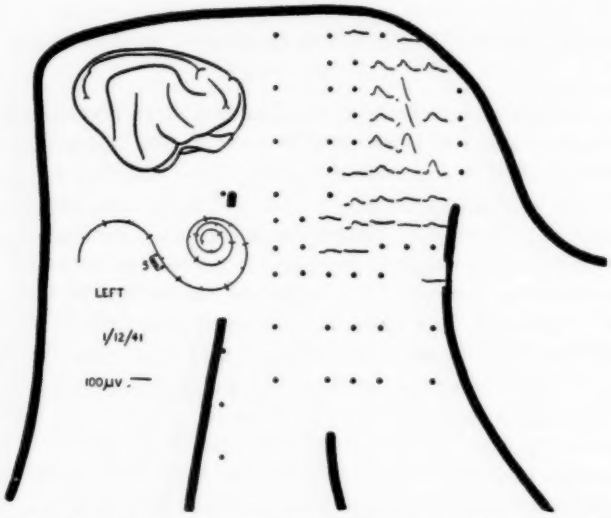

FIG. 2. Expt. 1/12/41; right cortex; left cochlea; stimulation of nerve fibers at free edge of osseous spiral lamina, 5 mm. from basal end. Responses are surface positive (positivity at "active" electrode recorded up, negativity down). For description of figure see text p. 319. For identification of sulci see Fig. 4. 4×.

behind the posterior ectosylvian sulcus, as was originally stated by Davis (1934), and by Gerard, Marshall and Saul (1936), in contrast to the reports of all others who have outlined the area by click stimulation.

In Fig. 4 the cortical areas of response to stimulation of the 6 mm. point on the basal turn and the 14 mm. point on the middle turn are illustrated. In this and the following experiment the amplitudes of the surface positive potentials were measured on the face of the

cathode ray tube and the values obtained were subsequently used in constructing the figures. In Fig. 4 the broken lines mark the limits of the responsive areas. Areas of maximal potentials are indicated in solid black. Note that the focus for the 14 mm. point lies anterior to the upper end of the posterior ectosylvian sulcus. A second and distinctly separate group of responses produced by stimulation of the 14 mm. point was encountered in the lower part of the auditory area

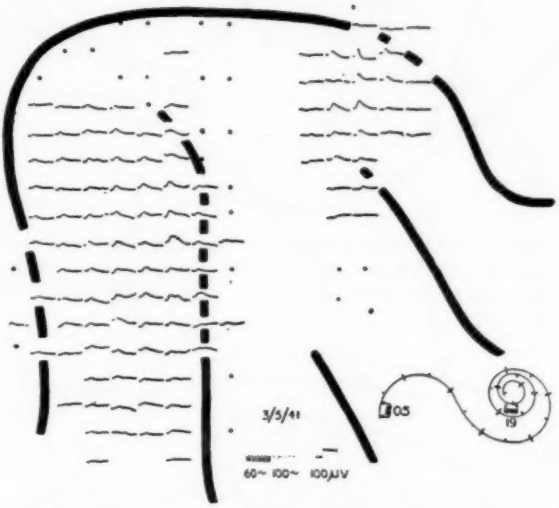

FIG. 3. Expt. 3/5/41; right cortex; left cochlea; stimulation of nerve fibers at 0.5 mm. and at 19 mm. from basal end. Position of response areas on lateral aspect of hemisphere shown in Fig. 9a. See text p. 320. 4×.

in the region marked out by the dashes. These are of especial interest and will be considered separately in section C.

In Expt. 2/5/41 nerve fibers were stimulated in sequence at 6 points on the left spiral, at 1.5, 4, 6, 8, 14 and 19 mm. from the basal end. The positions of the cortical areas in which potentials were evoked are shown in Fig. 5. It may be seen that the areas for the basal points overlap one another in an orderly manner but are separated by an interval from the area for the 14 mm. point. The latter, however, overlaps the area for the 19 mm. point. The cortex between the areas

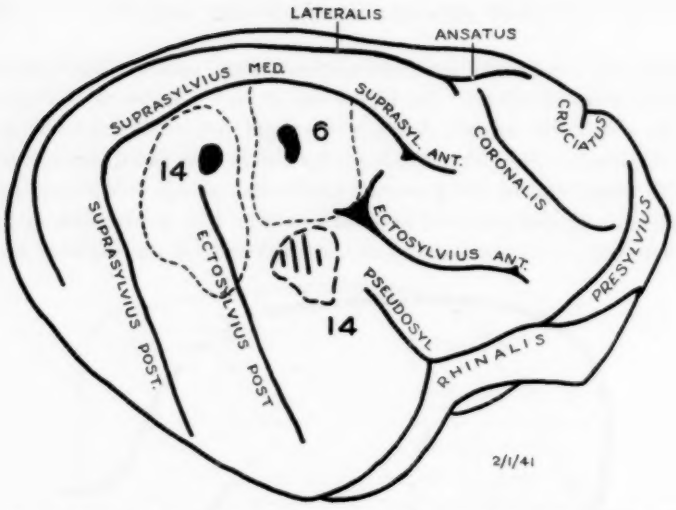

FIG. 4. Expt. 2/1/41; right cortex; left cochlea; stimulation of nerve fibers at 6 mm. (basal turn) and 14 mm. (middle turn) from basal end. Note that there are two response areas for the 14 mm. point. See text p. 320 and p. 327. Labels identify sulci. 2½×.

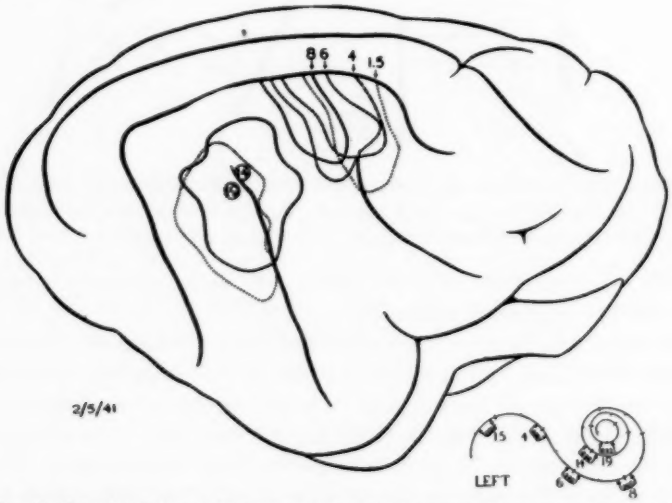

FIG. 5. Expt. 2/5/41; right cortex; left cochlea; nerve fibers stimulated at 1.5, 4, 6, 8, 14 and 19 mm. from the basal end of the spiral. See text p. 321. 2½×.

for the 8 and 14 mm. points undoubtedly is related to the portions of the basal and middle turns which were inaccessible to electrical stimulation.

It is evident from these and other experiments summarized in Fig. 10 that the cochlear nerve projects to the cerebral cortex in a definite anatomical pattern. Radiation fibers from the medial geniculate body apparently terminate in an over-lapping "laminated" system

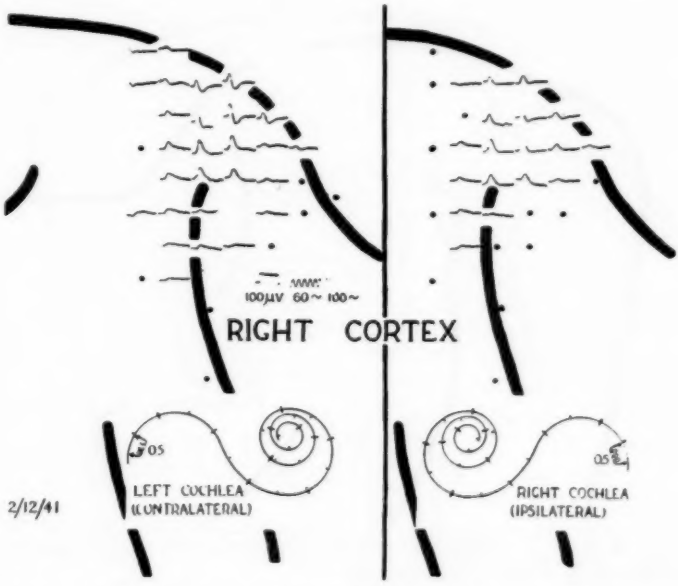

FIG. 6. Expt. 2/12/41; right cortex; left and right cochleae, stimulated at basal ends. Same area of right cortex activated by stimulation of either cochlea. Position of response area shown in Fig. 9b. See text p. 326. 5×.

in which functionally related groups of elements are oriented approximately in a dorso-ventral direction through the auditory area. The focal zone of this system lies in a band at right angles to the plane of lamination, along a line connecting the focal area for the basal end of the cochlea, which is situated at the upper end of the anterior ectosylvian sulcus, and that for the apex, which lies just behind the upper

end of the posterior ectosylvian sulcus. The cortical distance along this band, from the center of the focus for the basal end to the center of the focus for the apical end, is approximately one-half the length of the cochlear spiral in millimeters. At the basal end, at least, the focal area of response shifts 1 mm. along the focal band for each 2 mm. that the stimulating electrodes are moved along the edge of the osseous spiral lamina.

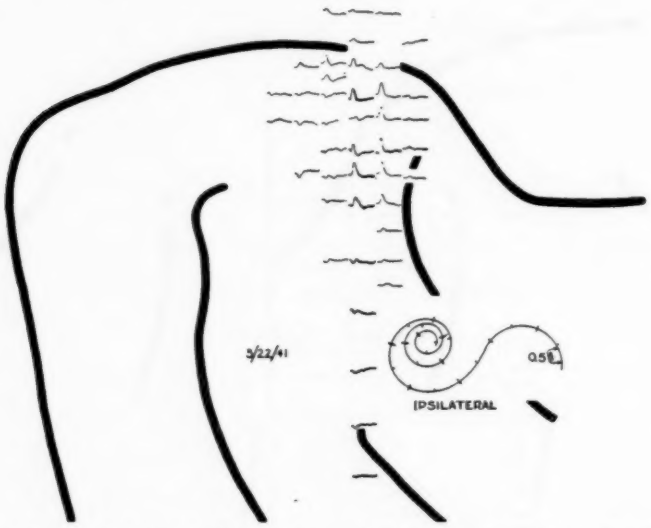

FIG. 7a. Expt. 3/22/41; right cortex; right (ipsilateral) cochlea; stimulated at basal end of spiral. Text page 326.

Inspection of the figures shows that the cortical areas for points on the apical and middle turns usually are larger than the areas for points on the basal turn. This may suggest greater cortical representation for the apex than for the base of the cochlea. However, it is evident from the dimensions of the cochlea that it should be easier to restrict electrical stimulation to a small group of fibers in the basal turn than to secure the same localization of stimulus at the apex. As will be pointed out in section C, we believe that the larger areas of response to apical and middle turn stimulations are due to excitation of larger numbers of nerve fibers.

B. Bilateral cortical representation of the cochlea

The experiments of Mettler, Finch, Girden and Culler (1934), of Brogden, Girden, Mettler and Culler (1936) and of Kemp and Robinson (1937) indicated that the crossed and uncrossed central connections of the auditory system in dogs and cats are of equivalent functional value. Bremer (1939), however, reported that the cortical

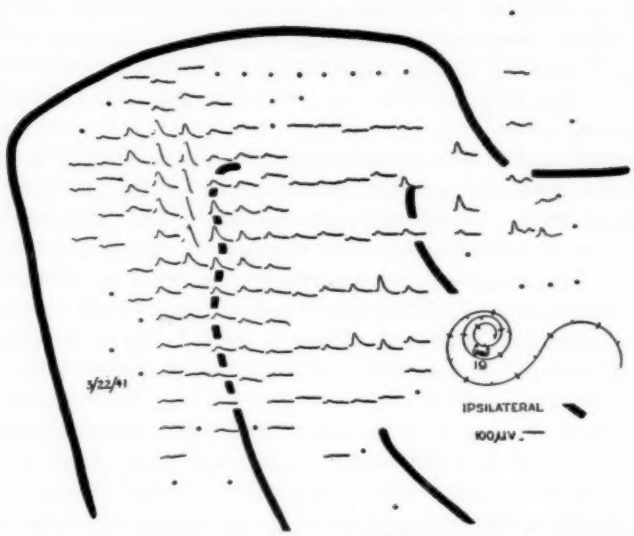

FIG. 7b. Expt. 3/22/41; right cortex; right (ipsilateral) cochlea; stimulated at apical end of spiral (19 mm. point). Note (1) primary response area with focus posterior to upper end of posterior ectosylvian sulcus (text p. 326) and (2) the second group of responses above the pseudosylvian fissure and in the anterior ectosylvian gyrus (text p. 327). Position of response areas of Fig. 7a and b, on lateral aspect of hemisphere, shown in Fig. 9c.

responses to click stimulation were slightly greater in the contralateral than in the ipsilateral hemisphere. Certain of our experiments have a bearing on this question. They indicate that each cochlea is represented about equally in both hemispheres, that impulses originating at symmetrical points in the contralateral and ipsilateral cochleae terminate in a common area of the cortex, and that the patterns of ipsilateral and contralateral representation are identical.

In Expt. 2/12/41 stimulating electrodes, connected through a selector switch to the source of stimulus, were placed on the basal end of each cochlea. The right cerebral cortex was exposed. For each placement of the "active" recording electrode records were made of the cortical responses to contralateral and to ipsilateral stimulation. Figs. 6 and 9b show that a common area received impulses from both cochleae and that the responses at each point within the area were similar in amplitude and latency. Actually the ipsilateral responses were slightly smaller than the contralateral ones, but since there is no way of knowing whether equal numbers of nerve fibers were stimulated in the two cochleae the difference cannot be considered significant.

Other evidence indicates that there is no obvious difference in amplitude of contralateral and ipsilateral response. In 3 additional experiments ipsilateral responses were studied and found to be of the same order of magnitude as those observed in contralateral experiments. This was also true of responses produced by electrical stimulation of the whole eighth nerve in 4 animals and of those evoked by click stimulation (Walzl and Woolsey, 1942b). In Expt. 3/22/41 the ipsilateral cochlea was stimulated at its basal and apical ends. The results are shown in Fig. 7a and b and in Fig. 9c. In amplitude the responses are as large as those observed on contralateral stimulation and the localization of the response areas is the same. Regarding the latter it should be noted that, in addition to the responses centered in the posterior ectosylvian gyrus of Fig. 7b, apical stimulation also evoked large potentials in the region above the pseudosylvian fissure and in the anterior ectosylvian gyrus. These will be given particular attention in the next section.

C. Double bilateral cortical representation of the cochlea

In the preceding sections we have been concerned with the pattern of localization in the primary cortical projection area of the auditory system and with the bilaterality of cochlear representation in the cortex. We shall now describe observations which indicate that, in addition to the primary projection system, there exists another system lying immediately ventral to the primary auditory cortex. This system is bilateral for each cochlea, is organized in a pattern inverse to that of the primary system and is recognizable not only by separate

anatomical localization but also by different physiological characteristics. Evidence for these statements is offered in Figs. 7b, 9c, 4, 8 and 9d and the accompanying discussion, and is summarized in Fig. 10.

In the preceding section attention was directed to the presence of a group of large potentials in the region above the pseudosylvian fissure and in the anterior ectosylvian gyrus (Fig. 7b and Fig. 9c) which were evoked by stimulation of the apex of the *ipsilateral* cochlea. On inspection one can distinguish this group of responses from the family of potentials centered in the posterior ectosylvian gyrus. The latter were of constant amplitude at any particular point and decreased in amplitude regularly in all directions from the center of maximum. In contrast, the responses at any one point in the pseudosylvian and anterior ectosylvian region varied markedly from one stimulation to another and the effect of this can be seen in Fig. 7b. At the boundary between the two areas responses were of minimal amplitude. This "second area of response" was also encountered in several, but not in all, experiments in which the *contralateral* apex was stimulated. It is significant that the region was not activated by stimulation of the basal coil although the primary projection area for the base lies immediately above it (cf. a and b of Fig. 7). That these responses resulted from stimulation of the auditory nerve was indicated by the fact that potentials in this area were evoked by click stimulation, and that these click responses were eliminated by lesions of the apical coil which abolished click responses in the posterior part of the auditory area. They remained after lesions of the basal coil which abolished responses in the anterior part of the auditory area without disturbing responses in the posterior part of the area (Walzl and Woolsey, 1942b). It appears, therefore, that they were responses due to stimulation of fibers in the apex of the cochlea.

In Expt. 2/1/41 (Fig. 4) a "second area of response" to stimulation of the 14 mm. point on the middle turn was noted. This area lay above and slightly posterior to the upper end of the pseudosylvian fissure and was distinctly separate from the primary area of response for this point. In another experiment involving the middle turn a similar area was seen overlapping somewhat more the "second area of response" for the apex.

Finally, the existence of an analogous "second response area" for

the basal turn is indicated in Figs. 8 and 9d (Expt. 1/4/41). In this experiment somewhat stronger stimuli than usual were employed. Fig. 8 shows the potentials evoked on stimulation of the basal end of the contralateral cochlea. The area of surface positive potentials is large and extends roughly in a diagonal manner across the cortex from the upper end of the anterior ectosylvian, where the responses are largest, through a region of minimal response to the lower posterior ectosylvian gyrus, where a second maximum occurs (Fig. 9d).

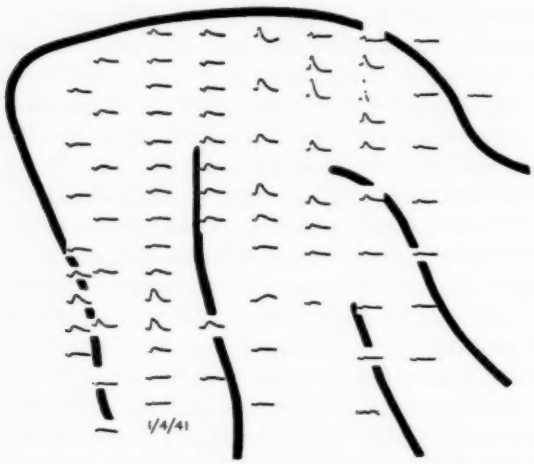

FIG. 8. Expt. 1/4/41; right cortex; left cochlea; strong stimulation of basal end of spiral. Cortical responses show two foci of large amplitude, separated by a region of low amplitude. For description see text p. 328. Position of response areas on lateral aspect of hemisphere shown in Fig. 9d. 4×.

From Figs. 7b, 9c, 4, 8, 9d and 10, it can be seen that the "second response areas" for the apical, middle and basal turns together form a band traversing the cortex immediately below the primary projection area. The pattern of organization in this system is inverse to that seen in the primary area (in the *primary system* the basal end of the cochlea is represented rostrally, the apex caudally; in the "*second*" *system*, the basal end is represented caudally, the apex rostrally). Whether this "secondary area" is related to the thalamus

by a separate projection system or is activated by relay from the primary area has not been determined in these experiments. The evidence from latency measurements is inadequate. Of course, both types of connection may exist. In the discussion, material from the literature is offered which indicates to us that the primary and "secondary auditory areas" are served by separate cortical projection systems.

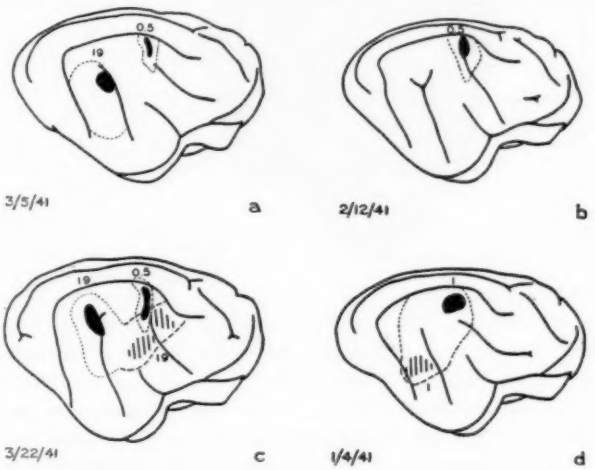

FIG. 9a. Shows position of response areas of Expt. 3/5/41 (Fig. 3) on lateral aspect of hemisphere. 1×.

FIG. 9b. Same for Expt. 2/12/41 (Fig. 6).

FIG. 9c. Same for Expt. 3/22/41 (Fig. 7a and b).

FIG. 9d. Same for Expt. 1/4/41 (Fig. 8).

It is significant that the responses in the lower posterior ectosylvian gyrus were elicited by stronger stimulation than usually was employed for excitation of the basal coil. This observation gives a clue to the interpretation of the experiments of this group. It appears that we are dealing with a system which not only projects to a different part of the cortex but also with one characterized by higher threshold, probably at some central synapse station. This suggestion is in keeping with the fact that stimulation of the apical and middle turns evokes potentials over larger areas of the cortex than does stimulation

of the basal end at similar intensity, presumably because restriction of stimulus to small groups of fibers at the apex is physically more

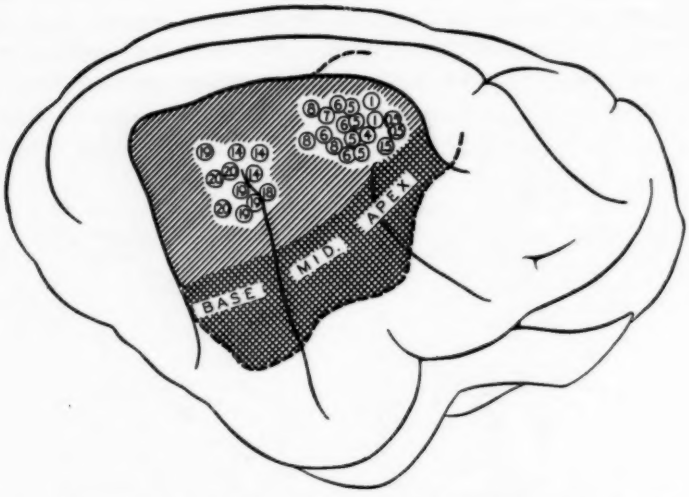

FIG. 10. Composite figure (2½×) made from data of all experiments (foci for one 19 mm. and one 1 mm. point, stimulated after figure was made, not shown). Circles with enclosed numbers indicate centers of primary foci of maximal response to electrical stimulation of nerve fibers at corresponding points (mm. from basal end) on the osseous spiral lamina of contralateral or ipsilateral cochleae. As illustrated in preceding figures, primary areas of response were generally elongated in a dorso-ventral direction. The interval without numbers, between the 8 and the 14 mm. foci, is probably related to the 9 to 13 mm. segment of spiral which was inaccessible to stimulation. Together the maximal foci form a focal band extending from the upper end of the anterior ectosylvian sulcus to the region just behind the upper end of the posterior ectosylvian sulcus.

Below the primary area, and overlapping it to a slight extent, lies a second area (cross-hatched) which is related to the cochlea in a pattern opposite to that found in the primary area. In the lower area the basal end of the spiral is represented caudally, the apical end rostrally.

Under light anesthesia responses were evoked at times in the area enclosed by the broken lines in the suprasylvian gyrus above the anterior end of the suprasylvian sulcus. This is the "motor" area for the pinna.

difficult than at the base. Excitation of more fibers at the apex would account for the more frequent association of "second area responses"

with apical than with basal stimulation. In 4 experiments in which the entire eighth nerve was stimulated and in several experiments in which the ear was stimulated by click (Walzl and Woolsey, 1942b), not only the primary but also the "second response area" was activated. These observations also suggest that we are dealing with a system in the lower part of the auditory cortex which is recruited to action only when many neurones of the peripheral nerve are excited.

DISCUSSION

A. Relation to other studies on the auditory cortex

The preliminary report on these experiments (Woolsey and Walzl, 1941) appeared nearly simultaneously with the initial accounts of two other studies dealing with the same general problem. Ades (1941b) found that small lesions in the medial geniculate body of the cat "eliminated click response in restricted parts of the auditory cortex, impaired response in adjacent parts and left response unaffected over the remainder of the area." Licklider (1941, and personal communication) reported a successful demonstration of frequency localization in the auditory cortex of the cat using pure tone stimulation and electrical recording. These studies have not been reported in full but it seems clear that both are consonant with our findings. More recently Ades (with Felder, 1942b) and Licklider (with Kryter, 1942) have extended their respective studies to the monkey with similar results. At the same time McCulloch, Garol, Bailey and von Bonin (1942) described "tonotopic" localization within the primary auditory cortex of the monkey and the chimpanzee.

The auditory area of the monkey lies within the Sylvian fissure on the dorsal aspect of the temporal lobe (Ades and Felder, 1942a). According to Licklider and Kryter (1942) "the lower frequencies produce their maximal effects in the antero-lateral part of the region, the higher frequencies in the postero-medial." In the cat, as we have shown, the primary cortical area for the apex (low frequencies) is situated caudally, that for the base (high frequencies) rostrally in the auditory area. This has been confirmed by Walzl and Woolsey (1942a) who made localized lesions in the organ of Corti and correlated the area in which click response disappeared from the cortex with the loss of response to limited parts of the frequency range as indicated

by cochlear potentials. Poliak's (1932) observation that the auditory radiation undergoes a spiral rotation through approximately 90 degrees in the monkey as compared with the carnivore indicates that the studies on cat and monkey are in accord. (See further discussion below.) Reports in the clinical literature (see Pfeifer, 1936), though not especially convincing, state that tones of higher frequency are appreciated in the medial division and those of lower frequency in the lateral part of the hearing center of man, and concur, therefore, with the experimental results on monkeys.

When this paper was presented in meeting, Dr. Lorente de Nó commented on the considerable degree of localization encountered at the cortical level. In view of the enormous complexity of the known connections of the peripheral neurones in the cochlear nuclei (Lorente de Nó, 1933b) and the possibilities for avalanche conduction in the nervous system, localization of such a degree at the cortex requires that afferent impulses be channelled through central neural structures by physiological mechanisms. Elsewhere (Lorente de Nó, 1934) he has expressed it thus: "due to the threshold of the neurons only a limited number of anatomically possible transmission pathways are accessible to the afferent impulses. The role of the central stations will consist in concentrating the stimuli in a few conductors rather than in increasing the number of active conductors." Certain physiological factors concerned in this channelling process recently have been considered at length by Marshall and Talbot (1942). It may be assumed that in the auditory system similar factors are at play.

On comparison of our results with those recorded in the literature it is clear that the total auditory area, as defined by electrical stimulation of cochlear nerve fibers, is larger than that generally outlined by click stimulation. It also seems clear from the literature that our total area of response must include more than one cytoarchitectonic area[4] (Campbell, 1905; Winkler and Potter, 1914; Kornmüller, 1933 and 1937; Bremer and Dow, 1939). Kornmüller and Bremer and

[4] Several of the brains used in the present study are being prepared for cytoarchitectural study and in addition the relations of the thalamus and cortex are being reexamined by the method of retrograde degeneration after lesions placed in accord with the results of the present investigation. Reports on these will be made later by Dr. J. E. Rose and the present authors.

Dow correlated their areas of click response with a region of distinct cytoarchitectonic structure. The gross extent of this area agrees in general with that of our primary projection system but it does not extend far enough caudad to include the focal area for the apex. Ades' (1941a) physiological observations with click stimuli and his Marchi study of fiber degenerations to the cortex following localized geniculate lesions tend to agree with the results of Kornmüller and of Bremer and Dow. Our "second area of response" lies below the cytoarchitectonic area of Kornmüller and of Bremer and Dow. It apparently was activated in the experiments of Davis (1934) and of Gerard, Marshall and Saul (1936) who reported auditory responses in the lower posterior ectosylvian gyrus. Ades (1941a) has suggested that some of the differences in the literature may be due to individual variations in the animals. Certainly fissuration of the brain does differ somewhat from cat to cat, as can be seen in the accompanying figures. In our experience, the character and the intensity of the click stimulus significantly influence the result. The intensity factor appears to be especially important for activation of the "second response area."

B. Relation of cortical areas to geniculate subdivisions

We cannot say on the basis of our experiments alone whether the "second response area" is activated by a separate projection system to the cortex or whether it represents a relay system to this region from the primary projection area. Of course, both types of connection may exist. However, the anatomical study of Waller (1939–40), when interpreted in the light of the present experiments, suggests that there is a separate projection system and that it originates in the large-celled division (pars magnocellularis of Rioch, 1929) of the medial geniculate body. At the same time his evidence indicates that the primary projection area takes origin from the small-celled division (pars principalis, Rioch). Finally, by comparison of his results with the pattern of cochlear representation in the cortex it is possible to deduce the probable pattern of cochlear representation in the medial geniculate body (cf. Ades, Mettler and Culler, 1939). The evidence in support of these statements may be summarized as follows with the aid of Fig. 11, which was assembled from Waller's illustrations. The experiment numbers are those assigned by him.

(a). Lesions restricted to our primary projection area (123 and 124R) caused degenerations only in the small-celled division of the medial geniculate body.

(b). Degeneration of the large-celled division occurred only in association with lesions involving the lower part of the auditory area (121, 127, 124L and 125).

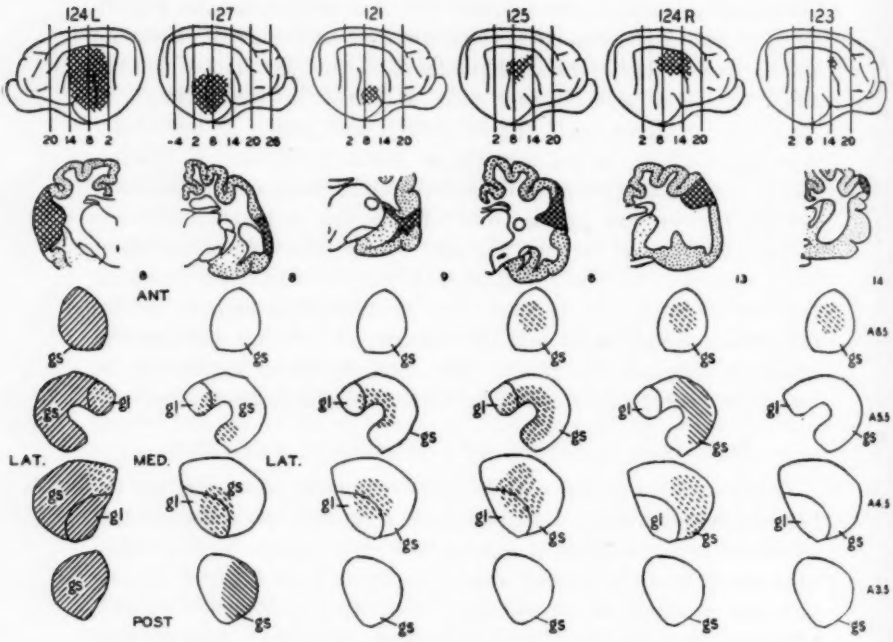

FIG. 11. Degenerations produced in small- (gs) and large-celled (gl) divisions of the medial geniculate body by lesions in the auditory cortex of the cat. Assembled from figures and text data in paper by Waller (1939–40). For description see text pp. 333–336. Numbers at top of figure are those of Waller's experiments. The brain drawings show surface extent of lesions and the cross-section diagrams illustrate depth of lesions at levels of section indicated by the accompanying numbers. The levels of geniculate section are indicated at the right. The cross-sections are vertical; A 6.5 is rostral; A 3.5 is caudal.

In 125 the lesion involved only the upper border of the "second response area" but was deep enough to cut off fibers from a larger portion of this region.

(c). Degeneration occurred in the rostral end of the small-celled division when a cortical lesion was placed at the basal end of the primary projection area (123).

This part of the nucleus degenerated in all other experiments in which the cortical lesions involved the basal end of the primary projection area (124R, 124L and 125); but it failed to degenerate in those experiments in which this part of the cortex was not destroyed (121 and 127).

(d). Degeneration of the caudal part of the small-celled division occurred only after lesions involving the posterior ectosylvian gyrus and the focal area for the apex, thereby suggesting that the apical end of the cochlea is represented in the caudal part of the small-celled division (124L and 127).

(e). Lesion of the upper part of the primary projection area caused degeneration in the outer part of the small-celled division (124R).

(f). Lesion of the lower part of the primary projection area caused degeneration in the inner part of the small-celled division (125).

(g). Lesion of the cortex in the "second response area" for the middle turn caused degeneration in the dorso-lateral part of the large-celled division (121). The lesion in this case was deep and may have interrupted fibers from the lower part of the primary projection area thus causing, in addition, degeneration in the medial part of the small-celled division.

(h). In Expt. 127 a lesion destroyed the "second response areas" for the basal and middle turns and involved the posterior part of the "second response area" for the apex; it also destroyed parts of the primary area for the middle and apical turns. In the large-celled division the degenerated area includes not only the dorso-lateral but also the postero-ventral and a small portion of the antero-dorsal part of this division.

(i). In 124L the lesion destroyed practically all the auditory cortex except the anterior part of the "second response area" for the apex. The only part of the large-celled division which did not degenerate completely was the antero-dorsal part.

Although the evidence just stated is not sufficient to be conclusive, it suggests the following tentative deductions which may be tested experimentally:

(1). The primary projection area receives its fibers from pars principalis (Rioch) of the medial geniculate body.

(2). The rostral (basal) end of the primary projection area is related to the rostral part of pars principalis, while the caudal (apical) end of the primary projection area is related to the caudal part of this division.

(3). The dorsal part of the primary projection area is related to the outer part of pars principalis; the ventral part of the primary projection area is related to the inner part of pars principalis, which is adjacent to pars magnocellularis.

(4). It appears that pars principalis must be "laminated" in such

a way that a lesion placed vertically in the primary auditory area of the cortex would result in a region of degeneration traversing the nucleus medio-laterally; a narrow rostro-caudal lesion through the center of the primary auditory area should produce a region of degeneration extending through pars principalis from rostral to caudal ends separating it into outer and inner parts.

(5). Pars magnocellularis of the medial geniculate body is related to the lower portion of the auditory area, i.e., to our "second response area." The postero-ventral part of pars magnocellularis projects to the posterior portion of the lower auditory cortex, i.e., to the ventral area related to the basal end of the cochlea; the antero-dorsal part projects to the anterior portion of the lower auditory cortex, i.e., to the apical portion of our "second response area;" the intermediate portion projects to the "second response area" for the middle turn.

Thus it appears that there is a "point-to-point" correspondence between the geniculate and cortical systems and that the inverse relationships of cochlear representation found in the primary and "secondary" systems of the cortex also exist in the medial geniculate body. The anatomical reversal must occur below the thalamic level. Therefore, as in the tactile system (Woolsey, Marshall and Bard, 1942), the organization of the auditory thalamus resembles that of the cortex rather than that of the segmental level. The bearing of this analysis on ablation experiments designed to test auditory localization in the cortex and in the thalamus need not be elaborated.

It is of interest to note that other instances of inverse relations in cortical systems have recently been reported. Talbot (1942) described a second functional localization in the cat's visual cortex lateral to the area striata and oppositely disposed. Garol (1942) reported a similar inversion on the motor side occurring in an area in which Adrian (1941) found a second somato-sensory area in the cat. The significance of these systems is not yet apparent.

C. On the probable location of a "second auditory response area" in the monkey

As has been noted, the auditory area of the monkey lies on the upper surface of the temporal lobe, in a position imposed in the course of phylogeny by development of the Sylvian fissure. Within this area the low frequencies are represented antero-laterally, the high

frequencies postero-medially (Licklider and Kryter, 1942; McCulloch et al., 1942, and personal communication). The postero-lateral margin of the area, then, should correspond with the dorsal or supra-sylvian border of the auditory area of the cat. If there is a "second response area" in the monkey, it must lie deep within the Sylvian fissure. Although there is no physiological evidence, as yet, bearing on this question, the studies of Walker (1938) and others permit one to examine the matter from an anatomical angle.

The precise homologies of the medial geniculate bodies of cat and monkey are not entirely clear (Compare Rioch, 1929; Friedemann; 1911–12; Aronson and Papez, 1934; Clark, 1935–36; Walker, 1938; Rundles and Papez, 1937–38). However, Walker and Aronson and Papez describe large- and small-celled divisions in the monkey which may correspond to pars magnocellularis and pars principalis of carnivores (Rioch, 1929). According to Walker and Aronson and Papez large cells in the monkey are found in the dorso-medial portion of the nucleus, in a position which is somewhat more dorsal than in the cat, as the result of rotation of the nucleus about its longitudinal axis in phylogeny (Poliak, 1932). Careful study of Walker's experiments reveals that pars magnocellularis degenerated only after lesions which penetrated deeply into the Sylvian fissure, especially after injuries involving the depths of the fissure posterior to the level of the insula. On the other hand, pars principalis alone degenerated following lesions limited to the koniocortex (the area which apparently receives the primary projection system) and after injuries of the outer half of the superior surface of the temporal lobe. Minkowski (1923) described degeneration of the antero-dorso-median part of the left medial geniculate body in a monkey sacrificed 6 weeks after removal of the left posterior parietal gyrus and the cortex surrounding the posterior end of the Sylvian fissure. Rundles and Papez (1937–38), after study of the thalamic degenerations in the brains of a mangabey and of a baboon, in which there were bilateral temporal lobe lesions, made the following summarizing statement: "Both parvocellular and magnocellular elements of the medial geniculate nucleus have cortical connections. The magnocellular portion (parageniculate nucleus) is related to the cortex near the bottom of the Sylvian fissure, probably caudal to the main acoustic area."

In support of the above evidence we can report anatomical observa-

tions made on a monkey prepared for another study. In this animal the occipital and temporal lobes had been ablated bilaterally, and in addition the left posterior parietal gyrus had been removed. The insula, i.e., the cortex overlying the claustrum, remained on both sides. On the left all cortex of the temporal lobe and of the Sylvian fissure posterior to the insula had been removed. On the right the posterior parietal gyrus was intact to the bottom of the Sylvian fissure and there was a very small amount of koniocortex lateral to the posterior end of the insula. The medial geniculate body had completely degenerated on the left but on the right a small portion of the anterior end of pars principalis remained, together with much of pars magnocellularis.[5]

Of great interest in relation to the question of a possible "second auditory projection system" in the monkey is the description by Poliak (1932) of a special temporo-parietal (posterior Sylvian) receptive region (area x in his figures 2, 10, and 24) which is contiguous to the primary auditory area and receives afferent fibers "originating in the internal geniculate body or somewhere in the ventro-lateral nucleus or even in the hypothalamus."

It seems to us that these data from various sources together suggest a consistent picture, which may be examined by further experimentation. The primary auditory area of the monkey lies lateral to the insula on the middle third of the superior surface of the temporal lobe disposed in a diagonal manner with the apex of the cochlea represented antero-laterally and the basal end postero-medially. The primary area, according to our interpretation of Walker's results, is related to pars principalis in the same way that the primary area appears to be related to pars principalis in the cat, allowance being made for rotation of the nucleus about its longitudinal axis in the monkey. Lying more deeply in the Sylvian fissure and extending more posteriorly than the primary projection area, probably on both walls of the Sylvian fissure (Poliak), is an area, corresponding to our "second response area" in the cat, which receives projection fibers from pars magnocellularis of the medial geniculate body. The most posterior

[5] We wish to thank Dr. J. E. Rose, of the Neurological Laboratory, Phipps Psychiatric Clinic, the Johns Hopkins Hospital, for his kindness in examining the sections of this brain for us.

portion of this "second response area" would correspond to the most anterior part of the "second response area" of the cat and, therefore, should receive impulses from the apex of the cochlea. The fact that the "second response area" of the monkey appears to be stretched out along the Sylvian fissure is in keeping with the gross alterations in structure which occur in the formation of the Sylvian region.

D. Comments on the formation of the Sylvian fissure

If the above analysis is correct the following comments concerning homologies of the Sylvian fossa are pertinent. Holl (1899, 1900) concluded on the basis of comparative gross morphology that the anterior ectosylvian sulcus of carnivores becomes in primates the posterior limb of the Sylvian fissure and the superior limiting sulcus of the insula. This view, however, was strongly opposed by G. Elliot Smith (1903) who maintained that the superior limiting sulcus of the insula and the posterior Sylvian fissure are derived from the "true" suprasylvian sulcus, which he distinguished as separable from the posterior suprasylvian (his postsylvian sulcus). The latter, according to Smith, becomes the superior temporal sulcus of primates. These views of Smith have been accepted by Kappers, Huber, and Crosby (1936) and by Connolly (1936). According to our deductions regarding the monkey, a "second response area" for the apex probably lies on both walls of the Sylvian fissure posterior to the insula. In the cat the "second response area" for the apex lies astride the upper half of the anterior ectosylvian sulcus. These areas, then, may be considered tentatively as physiologically homologous. Although it has not been established that physiologically homologous areas have any necessary relation to homologous gross morphological features, we suggest that the upper half of the anterior ectosylvian sulcus of the cat corresponds to the posterior Sylvian fissure of primates, as originally was thought by Holl. It seems likely also that the lower part of the anterior ectosylvian sulcus becomes the superior limiting sulcus of the insula, not only because of its relations to tissue overlying the claustrum in the cat (Winkler and Potter, 1914), but also because such a relationship is indicated in general in the brains of carnivores and ungulates (Holl, 1899, 1900). In the recent paper of Rose (1942) on the sheep's cortex, the fissure labeled *sd* (dorsal branch of Sylvian

fissure), Fig. 20, apparently corresponds to the anterior ectosylvian of the cat. It has the same relationship, as in the cat, to the anterior end of the suprasylvian sulcus, and the relationship to Rose's parietal (Par.) cytoarchitectural field is similarly suggestive. This field probably corresponds to the cortical areas receiving auditory projection systems in the cat. The lower portion of this fissure (*sa*, anterior branch of Sylvian fissure) curves forward above the insula. In our opinion the two branches of this fissure constitute the analogue of the anterior ectosylvian sulcus of the cat.

If the anterior ectosylvian sulcus of the cat is identified in this manner with the Sylvian fissure of the monkey, it is evident that in development of the Sylvian area, growth pivots, so to speak, around the upper end of the anterior ectosylvian sulcus, and that the temporal lobe grows forward beneath the insula carrying the primary auditory area with it into the position actually found in the monkey. At the same time it appears that the upper end of the anterior ectosylvian moves posteriorly with development of the parietal lobe, pushing back and straightening out the suprasylvian sulcus. The latter, then, is represented in its entirety by the superior temporal sulcus and does not enter into the formation of the Sylvian fissure as suggested by Smith. Although the gross forms of the sulci thus are altered, it is noteworthy that the original close relations of the upper end of the anterior ectosylvian sulcus and of the anterior end of the suprasylvian sulcus are preserved in the relations of the posterior end of the Sylvian fissure and the upper end of the superior temporal sulcus. Whether or not these views concerning the formation of the Sylvian region prove correct, it seems reasonable that future comparative physiological studies should throw considerable light on problems relating to the comparative morphology of the brain.

SUMMARY

1. By electrical stimulation of small groups of nerve fibers at the edge of the osseous spiral lamina (surgically exposed), it has been possible to demonstrate a "point-to-point" projection of the cochlea to the cerebral cortex of the cat.

2. The *primary projection pathway* terminates at the cortex in an overlapping "laminated" system in which functionally related elements are oriented approximately in a dorso-ventral direction through the

auditory area. The focal zone of this system lies in a band at right angles to the plane of lamination along a line connecting the focal area for the basal end of the cochlea, which is situated at the upper end of the anterior ectosylvian sulcus, and that for the apex, which lies just behind the upper end of the posterior ectosylvian sulcus.

3. Each cochlea is bilaterally represented in the primary projection area, and impulses from corresponding points of each cochlea terminate in common areas of each hemisphere. Ipsilateral and contralateral representations for each cochlea appear to be equal.

4. Evidence is presented which indicates that there exists a "*second auditory area*" lying immediately ventral to the primary auditory cortex. Spatially it is organized in a pattern inverse to that of the primary system, with the basal end of the cochlea represented caudally and the apex rostrally. It can be recognized not only by its separate anatomical location but also by its higher threshold of excitation. This system also is bilateral for each cochlea.

5. Evidence from the literature, in conjunction with our observations, indicates that this "second auditory area" probably is served by a separate projection system drived from pars magnocellularis of the medial geniculate body and that the primary area receives its fibers from pars principalis of this nucleus. The probable pattern of functional organization in the medial geniculate body is discussed.

6. Suggestions, based on the literature, are offered concerning the probable location of a "second auditory area" in the monkey. The bearing of these suggestions on homologies of the Sylvian fossa is discussed.

7. Since the fibers of the cochlear nerve are distributed in an orderly manner to the organ of Corti, localized regions of which respond optimally to particular frequencies of the sound spectrum, demonstration of a "point-to-point" projection of these fibers to the cerebral cortex provides an anatomical basis for tonal localization in the cerebral mantle.

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THE EFFECT OF LOW OXYGEN TENSION UPON THE DEVELOPMENT OF EXPERIMENTAL TUBERCULOSIS[1]

ARNOLD R. RICH AND RICHARD H. FOLLIS, JR.

Department of Pathology and Bacteriology, The Johns Hopkins Medical School

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The tubercle bacillus, as is well known, is an aerobic bacterium. Loebel, Shorr and Richardson (1) found that the human type bacillus lacks the capacity to derive energy from anaerobic glycolysis, a mechanism possessed by so many other cells. When completely deprived of oxygen while on the surface of nutrient fluid medium, the bacilli lost in several days both their capacity to respire and their ability to reproduce when transferred to a favorable environment. Novy (2) and Corper (3) and their associates have demonstrated that, within certain limits, the rate and luxuriousness of the growth of the bacilli *in vitro* are directly proportional to the oxygen tension of the gaseous environment; and Kempner (4) has shown that the respiration of the bacilli is similarly affected by the oxygen supply. At an oxygen concentration of 14 volumes per cent of one atmosphere, which is approximately that of alveolar air, the respiration of the bacilli proceeded at about its maximum rate. A reduction of the oxygen supply to 8 volumes per cent resulted in a 20 per cent decrease in the bacterial respiration, and further reduction of the oxygen supply entailed a still more drastic inhibition of the respiration of the bacilli.

There are a number of circumstances which suggest that the oxygen supply may play an important rôle in determining the degree of multiplication of the bacilli within the body. It has, for example, been suggested that at least one of the beneficial factors in therapeutic pneumothorax may be the reduction in the oxygen supply to the collapsed lung, since in a collapsed lung the normal replenishment of atmospheric air is prevented. Besides, the lung is an organ that receives, for the most part, venous blood, and Corper, Lurie and Uyei (3) have shown that tubercle bacilli thrive far better in the gaseous tension of normal

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alveolar air than in that of venous blood. Adams and Vorwald (5) found that following either haematogeneous or bronchogenic experimental infection, much less tuberculosis develops in a lobe of a lung from which air is excluded by bronchial occlusion than in the remaining, aerated parts of the lungs.

Another circumstance suggestive of the influence of oxygen supply is the fact that tuberculous mothers rarely give birth to tuberculous infants, and this even when the placenta itself is involved in the tuberculous process. In common with others, we have seen a number of examples of this fact. Experimentally, Vorwald (6) has demonstrated the marked resistance of the guinea pig foetus to tuberculous infection, even when the bacilli are injected directly into the foetal body *in utero*. It is well known from the studies of Eastman (7) and others that the oxygen supply to the tissues of the human foetus is decidedly lower than that of the body after birth; and Barcroft, who studied the oxygen content of the heart's blood of the foetal guinea pigs in Vorwald's experiments, found it to be only 8.2 volumes per cent.

Kempner (4) has presented calculations, derived from *in vitro* studies of the respiration of the tubercle bacillus under different conditions of oxygen supply, which suggest that the decrease in the oxygen tension of the atmospheric air may be a factor in whatever beneficial effect high altitude may have upon tuberculosis.

Finally, there are a number of considerations which suggest that the amount of available oxygen in the different tissues may play a rôle in determining the degree to which they are involved by tuberculosis. These considerations have been discussed by Corper and his associates (8) and others.

Since all of the various circumstances mentioned above involve factors, other than oxygen supply, which might be responsible for the observed inhibition of the tuberculous infection, it was regarded as desirable to attempt to determine whether the development of tuberculosis can be influenced simply by decreasing the body's oxygen supply. David (9), in 1912, reported a beneficial effect of low oxygen tension upon experimental tuberculosis, but his protocols are hardly convincing. Singer (10), in 1929, was unable to observe any such effect, and he concluded that the oxygen supply has no influence upon the infection. Both of these investigators, however, exposed the animals to the

low oxygen tension for only a brief period of each day. The present experiments were designed to study the effect of continuous exposure to low oxygen tension. For this purpose a chamber was constructed in which animals could be kept continuously at approximately any desired oxygen tension, with no alteration of the atmospheric pressure.

The chamber, which is a modification of other similar gas chambers, consists of a box and cover of galvanized iron (Fig. 1). Two plate glass windows, are set into one wall, 7 in. above the floor. A gutter 1 in. wide and 2 in. deep extends around the top of the box; into this the cover fits. The cover has a flange at right angles, 1.75 in. in depth. The gutter is filled with mineral oil to a depth of 1 in., so that when the cover is in position an air-tight seal is formed. Two hasps hold the cover in place. There are two holes (A and B), 0.25 inches in diameter, at opposite sides of the box, one 37 in. and one 10 in. above the floor. Brass tubing 2 in. long is soldered to these. A glass stopcock is attached by rubber tubing to A. B is connected to the oxygen system to be described below. A galvanized iron tray, 6 in. deep, fits snugly inside the box on the bottom. Wood shavings are placed in this, and are then covered by a grate of 0.75 in. mesh, that rests on legs 2 in. high. Two tiers of three trays, each measuring 5 in. in width and 1 in. in depth, extend along the entire length of the box on two sides, suspended by a flange that rests in the gutter at the top of the box. These trays are filled with soda lime (Wilson, 4–8 mesh). A small, rubber-bladed, automobile windshield fan attached to the upright rod of a ring stand, is placed in one corner of the box. This fan is connected with a storage battery by wire that runs through the oil seal of the cover. Food sufficient to last a week is placed in containers on the floor. Water is poured, as needed, into a receptacle immediately beneath the opening A, by removing the rubber tubing and stopcock from A and introducing a glass tube with an attached funnel through the opening.

The large spirometer of galvanized iron contains oxygen. The smaller reservoir contains nitrogen. The gasses are introduced from commercial tanks through inlets at the bases. The inner tank of the large reservoir is balanced by counterweights attached to a light rope running through pulleys. The nitrogen reservoir is not balanced. The oxygen spirometer is connected by rubber tubing to a glass tube inserted into the middle opening of a Woulff bottle half filled with water. This tube is arranged so that gas bubbles through the water when slight negative pressure is present in the animal chamber. A water manometer is attached to the second opening of the Woulff bottle. The third opening is connected with the opening B of the chamber by tubing.

The routine procedure was as follows. Animals were placed in the box, the lid put in place, and the fan turned on. Nitrogen was introduced into the box through the lower opening (A) while the upper one (B) was disconnected. Three reservoirs full of nitrogen were necessary

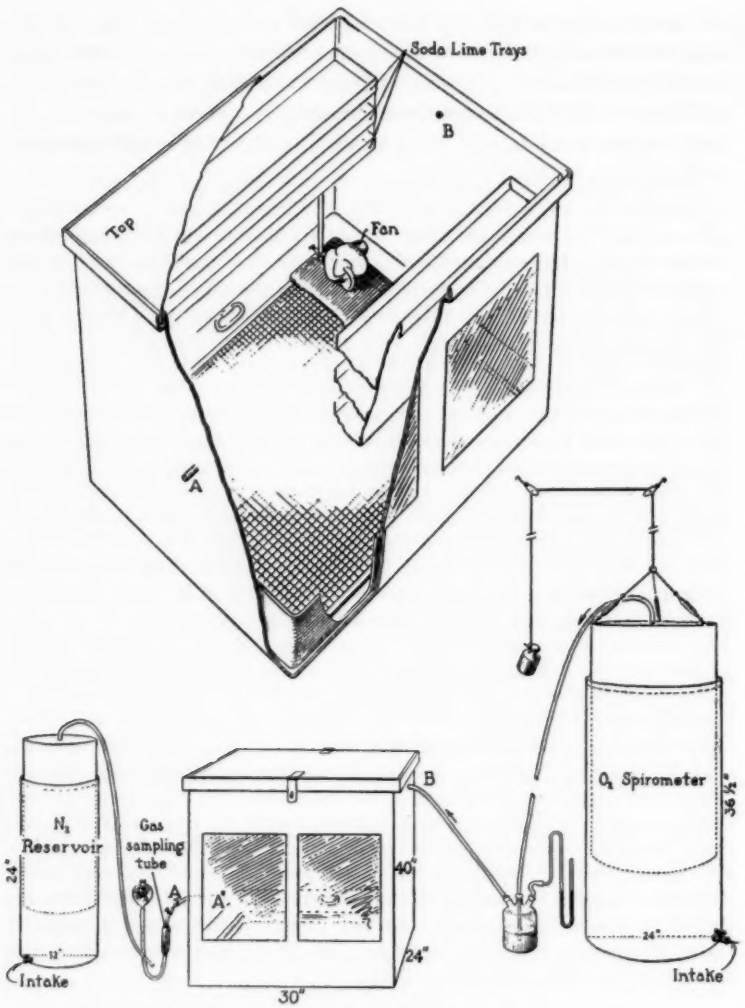

FIG. 1. DIAGRAM OF THE GAS CHAMBER

to bring the concentration of oxygen in the box down to approximately 10 volumes per cent. When the desired oxygen content was attained in the chamber (and this could be determined only by trial and samp-

ling), the nitrogen reservoir was disconnected, and the oxygen system reconnected. The carbon dioxide given off by the animals was absorbed by the soda lime; this, together with the oxygen utilization, created a slight negative pressure in the chamber which drew oxygen in from the large spirometer. The environmental temperature was kept as constant as possible, in order to avoid changes in volume of gas in the chamber. Samples of gas were taken with an ordinary sampling tube from the opening A, and were analyzed for their oxygen and carbon dioxide content in an Orsat-Henderson gas analysis apparatus. The analyses were made daily, once or as often as indicated. If the oxygen content of the chamber fell below the desired level, some of the gas mixture was withdrawn from the chamber by a suction bottle attached to opening A. This drew oxygen into the chamber from the spirometer by creating a negative pressure. Except in the first three experiments no attempt was made to absorb water vapor from the chamber. In those experiments, sulphuric acid was placed in containers on one of the soda lime trays, but it was not effective. The factor of moisture was controlled by an experiment in which the animals were subjected to all of the conditions of the chamber except low oxygen tension, as will be mentioned below. In all experiments the chamber was opened once weekly for cleaning and replenishing the food and soda lime. At such times the animals were exposed to the oxygen content of the room for about 15 minutes. 15–30 additional minutes were required to bring the oxygen concentration within the chamber to the desired level. If an animal died the chamber was opened in order to remove it, and was then quickly closed and the chamber oxygen brought back to the original level.

Nine experiments were carried out, using a total of 106 animals, of which 13 were rabbits, averaging 1500 grams in weight, and 93 were guinea pigs, weighing 400 to 600 grams. In 5 of the experiments the oxygen content of the chamber was maintained at approximately 9–10 volumes per cent; in 2 experiments at approximately 12 volumes per cent; in 1 experiment, at 13–14 volumes per cent; and in 1 experiment, at 20 volumes per cent. In each experiment a group of guinea pigs or rabbits were infected with equal and relatively large numbers of virulent tubercle bacilli. Half of the group were kept continuously in the chamber, and the remaining half were kept as controls in open cages in the same room. All were fed a balanced commercial prep-

aration ("Domino All-In-One Rab-ets"). The guinea pigs received carrots in addition.

The tubercle bacilli were carefully ground in a mortar with a drop of sterile bile. 5 to 6 cc. of physiological saline were added slowly, drop by drop. The suspension was centrifugalized for two minutes at low speed, and a loopful of the supernatant was then stained with Kinyoun's stain. If clumps were still present the suspension was filtered through coarse filter paper. The suspension was diluted until the desired number of bacilli (20–30) per oil immersion field was obtained. The rabbits were infected intravenously with 0.5 cc. of a suspension of virulent bovine bacilli (Ravenel strain). The guinea pigs were infected subcutaneously with 0.1 cc. of a virulent human type bacillus obtained from a case of pulmonary tuberculosis. In each experiment the control and test animals were matched by weight, and test and control animals were injected alternately. The test animals were placed in the chamber immediately after infection. The controls were placed in wire cages in the same room.

Since more guinea pigs than rabbits could be kept in the chamber at one time, after the initial experiments guinea pigs were used exclusively. Occasional animals died of pyogenic pneumonia. When a chamber animal died after three weeks sojourn in the chamber, a control was killed for comparison. Each experiment was terminated after a period of three or four weeks. All of the animals were killed, careful autopsies were performed to determine the amount of macroscopic tuberculosis, and sections were made of representative portions of the spleen, liver, both lungs, and bronchial lymph nodes. The sections of each lung passed through the entire organ, from apex to base and from lateral pleura to hilum. The sections of spleen included the entire length and breadth of the organ. The grading, as presented in the charts, is based on the microscopic study. The slides of the sections of the organs of each animal were projected upon a screen by means of a microprojector, and were studied carefully to determine the number, extent and character of the lesions, which were graded from zero to 5 according to the degree of involvement. Especial precautions were taken to insure that the study of the sections was carried out with no knowledge of the type of animal under scrutiny (*i.e.*, whether chamber or control) until the grading of all of the animals of the experiment had been completed. Each experiment was graded twice in this manner,

and in instances in which the two gradings of an organ did not agree, a third examination was made.

TABLE I

Experiment VII. Oxygen 10 Vols. Per Cent

	GUINEA PIG	SPLEEN	LIVER	LUNG	TOTAL	TOTAL SPLEEN	TOTAL LIVER	TOTAL LUNG	TOTAL	AVERAGE TOTAL
Chamber	490B	1	0	0	1					
	495B	2	0	0	2					
	500B	2	0	0	2					
	481B	2	0	0	2					
	486B	2	1	0	3					
	489B	1	1	0	2					
	496B	1	0	0	1					
	480B	1	0	0	1					
	497B	1	0	0	1	13	2	0	15	1.6
Controls	492C	3	3	3	9					
	471C	4	2	1	7					
	484C	4	4	2	10					
	485C	3	2	2	7					
	482C	2	1	0	3					
	499C	3	2	1	6					
	488C	2	1	2	5					
	498C	3	2	0	5					
	493C	4	3	2	9	28	20	13	61	6.8

TABLE II

Experiment V. Oxygen 9–10 Vols. Per Cent

	GUINEA PIG	SPLEEN	LIVER	LUNG	TOTAL	AVERAGE SPLEEN	AVERAGE LIVER	AVERAGE LUNG	AVERAGE TOTAL
Chamber	403B	1	0	0	1				
	416B	2	0	0	2				
	420B	1	2	0	3				
	410B	1	0	0	1	1.5	0.5	0	1.7
Controls	428C	4	4	2	10				
	436C	3	4	2	9				
	423C	5	5	3	13				
	435C	4	3	2	9				
	406C	3	3	1	7	3.8	3.8	2.0	9.6

Briefly, the results of these experiments showed that when animals were kept continuously in the chamber, exposed to an oxygen content

not higher than 9 to 10 volumes per cent, the development of the experimental infection was markedly inhibited in comparison with the controls kept in open cages in the same room. That the inhibition of the disease was due to the low oxygen tension and not to other factors associated with the sojourn in the chamber (*e.g.*, differences in humidity between the chamber and the room outside) seems clear from the fact that in one experiment the oxygen content of the chamber was kept at 20 volumes per cent (*i.e.*, that of atmospheric air), all other conditions remaining the same as in the experiments with low oxygen tension. In this control experiment there was no difference whatever between the amount of tuberculosis in the eleven chamber animals and the ten in the open cages. Furthermore, in experiments in which the oxygen content of the chamber was kept at 12–14 volumes per cent, the inhibitory effect was greatly diminished. While at these latter levels of oxygen supply there was a difference between the amount of tuberculosis in the chamber animals and the controls detectable by the above method of grading, it was so slight as to be scarcely significant. Tables I and II illustrate the marked inhibition of the infection produced by a reduction in the oxygen content of the chamber to 9–10 volumes per cent. Figs. 2–7 illustrate the tissues of a chamber and a control animal.

DISCUSSION

These experiments demonstrate that, in the experimental animals used, the progress of tuberculous infection can be markedly inhibited by restricting the oxygen supply to the tissues. This is in keeping with previous studies on the inhibitory effect of reduced oxygen supply upon the respiration and multiplication of the tubercle bacillus *in vitro* (1) (2) (3) (4). In the present studies it has required a drastic reduction of the oxygen in the surrounding atmosphere (a reduction to about 50 per cent of the normal atmospheric oxygen) in order to produce a marked inhibitory effect upon the bacilli within the tissues. Nevertheless, the experiments demonstrate that if the oxygen supply to the bacilli within a lesion is sufficiently decreased, the activities of these strict aerobes will be greatly inhibited.

It must be borne in mind that the oxygen available to the bacilli within lesions is much lower than that of atmospheric air. The oxygen

tension of atmospheric air is 20 volumes per cent (159 mm. Hg.); that of the alveolar air in the lungs is only 14 volumes per cent, or 100 mm. Hg., which is the maximum that will be carried by the blood to the tissues under normal conditions when the body is at rest. However, much of this oxygen is seized by the tissue cells for their own use; and in the case of a tuberculous lesion, the accumulated inflammatory cells will also take up oxygen. Loebel, Shorr and Richardson (1) and others have demonstrated that the mononuclear phagocytes, which are the essential cells of the tuberculous lesion, have a very active oxygen consumption. In their studies, the respiration of these phagocytes was found to be twice as great as that of dog's muscle. In non-necrotic tuberculous lesions, therefore, the amount of oxygen that will be available for the tubercle bacilli within the lesions will be curtailed by the oxygen consumption of the phagocytes of the lesion. Loebel, Shorr and Richardson (1) present calculations which led them to conclude that the rate of oxygen consumption of the mononuclear phagocytes is such that, with a normal oxygen content of alveolar air (14 volumes per cent), there will be a complete lack of oxygen in the center of a visceral tubercle when its radius is between 0.075 and 0.35 mm. Tubercle bacilli in the center of such a tubercle would therefore die, since they cannot exist without oxygen. The mononuclear phagocytes in the anoxaemic center of the tubercle could remain alive because of their anaerobic glycolytic mechanism, which the tubercle bacillus lacks. We need not accept the above calculation as being necessarily completely applicable to actual *in vivo* conditions, but it is instructive in emphasizing the fact that even under normal conditions of atmospheric oxygen the tubercle bacilli within lesions are faced with a constant competition with fixed tissue and phagocytic cells for oxygen, which is essential for their respiration and multiplication. Under conditions of lowered atmospheric oxygen tension, as in the present experiments, the bacillus will be at a correspondingly greater disadvantage as regards oxygen supply, since less oxygen will be carried to the tissues. To what extent oxygen diffuses into inspissated caseous material is not known. It may well be that oxygen lack is a factor in the familiar disappearance of bacilli from the centers of caseous lesions.

The present experiments support the view that low oxygen tension

can be an important factor in the beneficial effect of collapse therapy in pulmonary tuberculosis; and they likewise support the view that the relative anoxaemia of the foetus is a factor in the resistance of the foetus to tuberculous infection, as mentioned in the beginning of this paper. They offer, however, little support for the view that the decreased oxygen tension in the atmospheric air at high altitude resorts can inhibit significantly the activities of the tubercle bacillus—at least not at the altitudes at which sanatoria are situated, for it required a much lower oxygen tension to affect the infection significantly in our experimental animals. For example, the oxygen tension of the atmospheric air at Denver, Colorado (5,000 ft.) is 17.25 volumes per cent, and at Davos, Switzerland (5,300 ft.) it is 17 volumes per cent. An oxygen tension of 10 volumes per cent, which was the level necessary for a significant effect on the infection in the present experiments, is equivalent to that in the atmosphere at 19,000 ft. The highest permanent community in the world is the small group of mine workers at 'Quilcha, Chile (17,500 ft.). It is therefore unlikely that the degree of atmospheric oxygen tension at high altitude resorts will, in itself, affect the infection significantly. It is conceivable, however, that the lowered oxygen tension in the air at high altitude resorts, acting in conjunction with the low oxygen tension in a lung subjected to therapeutic pneumothorax, may exert a deleterious effect upon the tubercle bacilli in the collapsed lung.

In any consideration of the effect of oxygen tension at high altitudes it must be remembered that the compensatory mechanisms of the body (increased heart action; increased respiration; compensatory polycythemia) can neutralize the effect of the atmospheric oxygen deficiency. The degree to which these compensatory mechanisms operate in each individual body is important in determining the degree to which tissue anoxaemia will result from a given decrease in atmospheric oxygen. Haemoglobin determinations and red cell counts were made on the blood of a few of the guinea pigs exposed to low oxygen tension. At the end of two weeks sojourn in the chamber at 10 per cent oxygen, the red cells were increased 30 per cent and the haemoglobin 16.5 per cent above normal.

It is well known that in the tuberculous guinea pig the lungs become only moderately involved, whereas the liver and spleen become ex-

tensively diseased. In contrast, in the rabbit the lungs become massively involved, while the liver and spleen show little or no macroscopic tuberculosis. This has been widely attributed to some peculiar native immunity of the rabbit's liver and spleen to tuberculosis. A pointed study of the matter, however, reveals that the liver and spleen of this animal possess no inherent native immunity to the infection. If rabbits are killed at intervals following an intravenous infection it becomes evident that tuberculosis begins to develop in the liver and spleen quite as in the lungs; but as the lesions become more and more extensive throughout the lungs, crowding out the air spaces and leading to anoxaemia, the tubercles in the liver and spleen become arrested, undergo regression and disappear, until, at the time of death, very little evidence of tuberculosis remains in the latter organs (Figs. 8, 9, 10, 11). The regression of the lesions is particularly striking in the liver, doubtless because the very high oxygen consumption of the hepatic cells depletes the available oxygen to a greater extent than do the tissue cells of most other organs. These observations, together with the results of our experiments on the effect of a decreased oxygen supply, indicate that the so-called "native immunity" of the rabbit's liver and spleen is really a matter of alteration of oxygen supply, rather than of any inherent native immunity that is incompatible with the establishment of tuberculous lesions.

In view of the marked cellular activity of the constantly proliferating bone marrow, its oxygen consumption is, probably, likewise high; and as the pulmonary disease progresses in the rabbit, regression of the tuberculous lesions in the marrow, similar to that which occurs in the liver, was noted some years ago by Doan and Sabin (11).

Corper and his associates (8), to whom credit is due for emphasizing years ago the importance of considering the influence of oxygen supply as a factor in determining the fate of tubercle bacilli within the body, were driven by their studies of the degree of involvement of different organs to conclude that the mononuclear phagocytes of the different organs, and of the same organ in different species, are inherently different in their capacity to destroy tubercle bacilli. There is no acceptable evidence for that view. It is now well established that many of the mononuclear phagocytes that make up tuberculous lesions are derived from the blood stream, and there is no evidence that the

mononuclear cells situated in the various tissues differ functionally from each other or from those in the circulation. The present experiments demonstrate that if the guinea pig's liver, in which tuberculosis characteristically advances to a marked degree, is subjected to a reduced oxygen supply, the progress of the hepatic disease is restricted in as marked a degree as it is in the rabbit, in which anoxaemia occurs spontaneously as a result of the more marked pulmonary involvement. The reason for the more marked involvement of the rabbit's lung need not be discussed here further than to mention that, as was pointed out by Krause (12), one factor may be the circumstance that the lymphatic tissue of the rabbit's lung is scattered throughout the lung substance, instead of being concentrated in extrapulmonary hilar nodes, to which bacilli deposited in the lung can be drained, as in the guinea pig and most other animals.

That tuberculous lesions frequently progress in the kidneys of the rabbit while regression is taking place in the liver may well be related to the circumstance, demonstrated by Levy and Blalock (13) in the dog, that the renal blood flow is high, and the oxygen consumption of the kidney very much lower than that of the liver. Tubercle bacilli, therefore, have a much greater opportunity to obtain oxygen in the kidney than in the liver. It hardly need be stressed that, while the present studies demonstrate that oxygen supply can exert an important influence upon the progress of tuberculous infection, it is by no means the sole important factor. This is indicated by the fact that progressive tuberculosis is a great rarity in the guinea pig's kidney, even though there is no present evidence that the oxygen supply is any less abundant than that of the rabbit's kidney in which progressive lesions commonly occur. Circumstances of this sort emphasize the need for a search for other local factors which influence the ability of the tubercle bacillus to survive and to multiply within the tissues.

SUMMARY

The progress of tuberculous infection was markedly inhibited in animals infected with virulent tubercle bacilli and kept continuously in a chamber at low oxygen tension.

While additional factors are, undoubtedly, highly important in determining the progression or retrogression of tuberculous lesions, the

present studies indicate that the amount of available oxygen in the tissues, and the degree to which different types of lesions permit the diffusion of oxygen, can play a rôle of no small moment in determining the degree to which tubercle bacilli will multiply and cause the progression of lesions in the infected body. The relation of these studies to the beneficial effect of collapse therapy, to high altitude resorts, and to the high resistance of the foetus to tuberculous infection, is discussed.

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EXPLANATION OF ILLUSTRATIONS

(Photographs by Mr. Milton Kougl)

FIG. 2. Lung of chamber guinea pig, Experiment V, 10% oxygen. No tuberculosis found grossly or microscopically.

FIG. 3. Lung of control guinea pig, Experiment V. Lesions of this type were scattered through the lungs, though not profusely.

FIG. 4. Bronchial lymph node of chamber guinea pig of Fig. 2. No tuberculous lesions found.

FIG. 5. Bronchial lymph node of control guinea pig of Fig. 3. The node is almost completely replaced by tuberculous tissue with caseation. The small, remaining *uninvolved* portions are indicated by arrows.

FIG. 6. Liver of control guinea pig of Fig. 3. There are diffusely scattered, often caseous, lesions of various sizes.

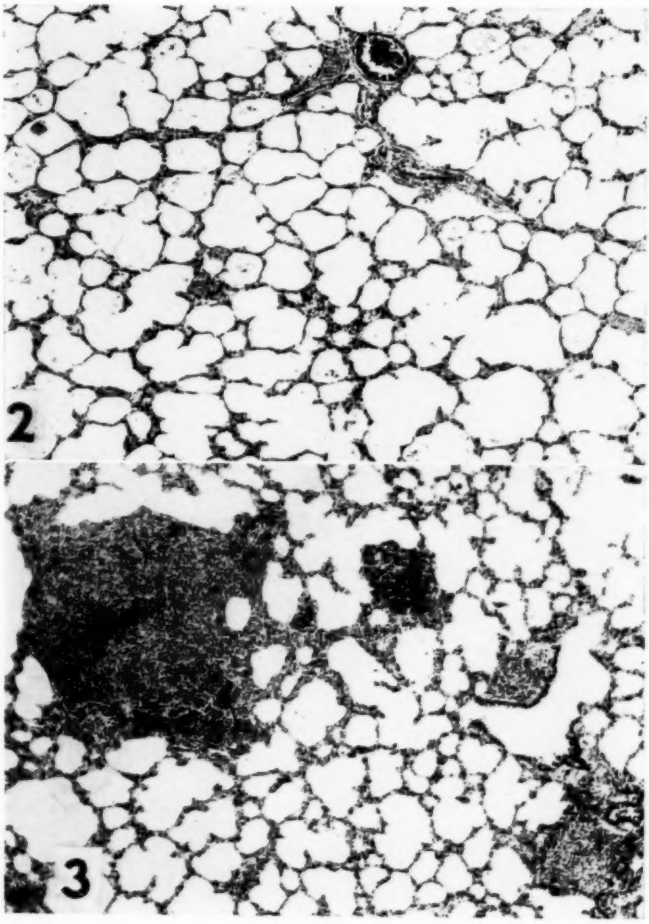
FIG. 7. Liver of chamber guinea pig of Fig. 2. No tuberculous lesions were found grossly or microscopically.

FIG. 8. Lung of rabbit 14 days after the intravenous injection of virulent bovine tubercle bacilli.

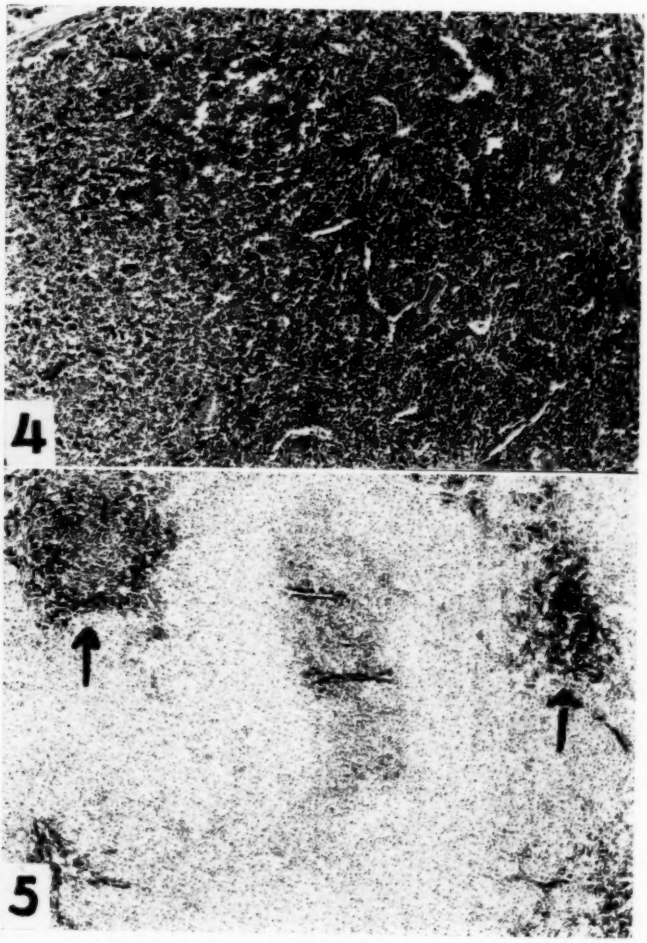
FIG. 9. Liver of same rabbit as in Fig. 8, showing that tuberculosis begins to develop in the liver as in the lung.

FIG. 10. Lung of rabbit, 21 days after intravenous infection with virulent tubercle bacilli in same dose as in rabbit of Fig. 8. The tubercles are large and more numerous, the lungs being thickly studded with them.

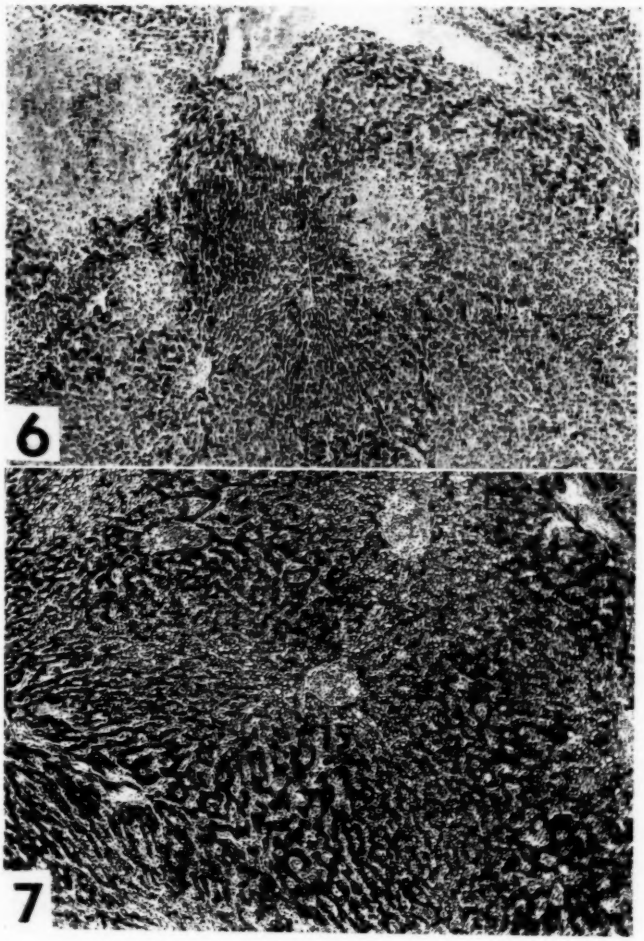
FIG. 11. Liver of same rabbit as in Fig. 10, showing the process of disappearance of hepatic tubercles concomitant with the reduction in the pulmonary air space. The arrows mark blood-filled holes, with occasional remaining epithelioid or giant cells. These holes are spaces from which tubercles have disappeared. In such areas the liver will be restored to normal.



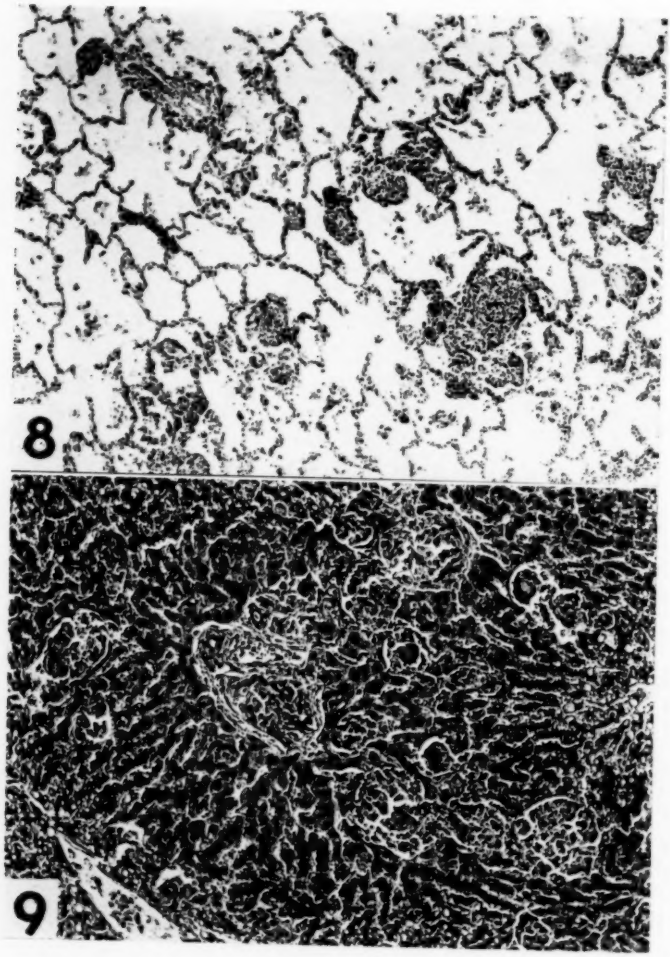
FIGS. 2 AND 3



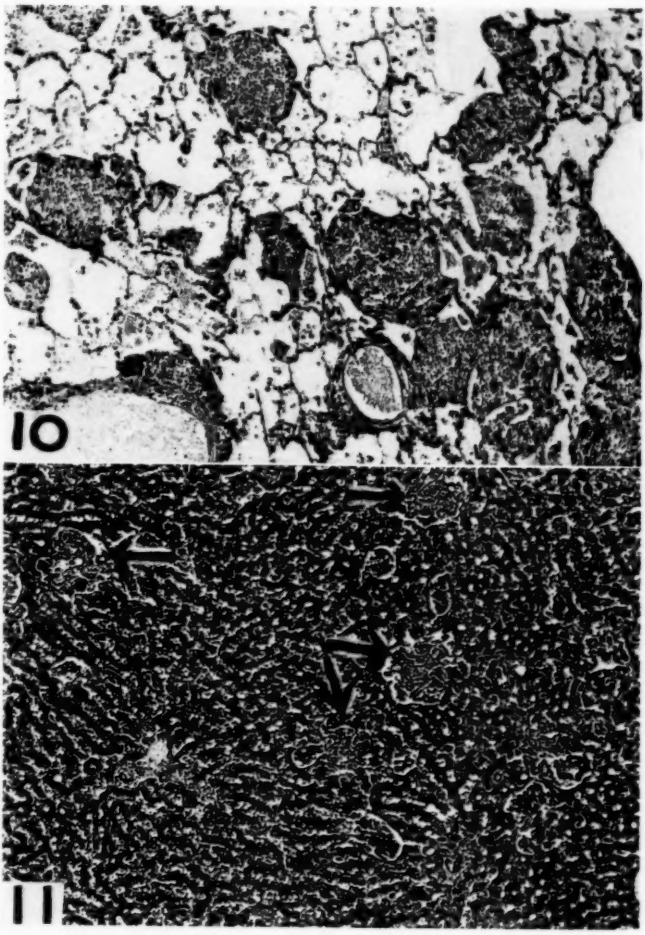
FIGS. 4 AND 5



FIGS. 6 AND 7



FIGS. 8 AND 9



FIGS. 10 AND 11

THE SERUM PROTEINS IN SARCOID: ELECTROPHORETIC STUDIES[1]

A. MURRAY FISHER, M.D., AND BERNARD D. DAVIS, M.D.

From the Medical Clinic and the Chemical Division of the Johns Hopkins Hospital and Medical School

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It is a well established fact that cases of Boeck's sarcoid usually exhibit an elevation of serum globulin; in fact this feature of the disease is considered an important point in its diagnosis. The recently refined electrophoretic method of Tiselius (1) now makes possible the accurate analysis of serum in terms of albumin and three globulin components. In making this study we were interested in trying to determine what part of the globulin fraction is increased, whether there is evidence suggesting that this increase is in any way specific for sarcoid, and whether or not the alterations in the serum proteins can be correlated with the activity of the disease.

Salvesan (2) in 1935 was the first to call attention to this abnormality. He found an increase in the total proteins of the blood in 3 cases of sarcoidosis, the values ranging from 9 to 9.67 grams per cent. These high values were due to an increase in the globulin fraction. Harrell and Fisher (3) and Harrell (4) found the total proteins above 8 grams per cent in all but 3 of 11 cases which they studied and the albumin: globulin ratio was reversed in all 8 cases during the active stage. Normal values were found in one of these after recovery. Fractional precipitation of the serum was done in 3 cases and the patterns were found much like those in hepatic cirrhosis. In the latter, however, there is often an increase in serum globulin without a rise in total proteins. Bing (5) found a rise in serum globulin in 2 of 4 cases of sarcoid. Gutman and his coworkers (6), using the Howe method of fractionation, found in 5 patients with sarcoid that the hyperglobulinemia was associated with an elevation of the "Euglobulin" and "Pseudoglobulin I". The experience with the disease to date at the Johns Hopkins Hospital is given in a recent report of 31 cases by Longcope

[1] The electrophoretic analyses were done by Dr. Davis and Mr. Edward Leszozynski.

(7). Serum protein determinations were made in 18 of these cases. The albumin fraction was below normal in all but one of the patients, whereas the globulin was above the average in all but one. The cases we are reporting here comprise part of Longcope's series.

Hyperglobulinemia has been found in a number of disorders other than sarcoid. It was reported by Bing (5) in various chronic bacterial infections including some cases of tuberculosis. The report by Gutman et al. (6) includes an analysis of this abnormality in the sera of 15 patients with miscellaneous infections. According to the Howe procedure the same fractions were increased as in the cases of sarcoid. It is likely that in a wide variety of chronic infections a rise of serum globulin will be found during certain stages of the illness. In lymphogranuloma venereum an elevation in the level of serum globulin is an important feature of the disease (6) (8), and here again (6) the same fractions have been found increased as have been noted in patients with sarcoid. In cirrhosis of the liver, one often finds a slight increase in the globulin fraction while the serum albumin is reduced (4) (5) (6). Kala-azar is an example of a parasitic infection with hepatic involvement, and it is well known that in patients suffering from this disease the serum globulin is high. In multiple myeloma serum globulin is usually markedly elevated. Electrophoretic studies (6) in patients with this disease revealed usually an increase in either the gamma globulin or the beta globulin although the results varied greatly in different patients. These are only a few examples of conditions in which hyperglobulinemia is often found. It is possible that by electrophoretic analysis specific patterns may be found for some of these conditions.

This report deals with 12 cases of sarcoid in various stages of the disease, and includes observations on 3 normal controls.

METHODS

The electrophoretic analyses were performed by means of the Longsworth (9) modification of the apparatus of Tiselius which has by now been used so widely in the study of clinical material that it requires no description here. In most studies the serum has been dialyzed against a phosphate or barbiturate buffer at pH 7.4 or 7.7 which yields a pattern of albumin (A) and three or four globulins (alpha, one or two betas, and gamma). Following the suggestion of Longsworth we have been using a more alkaline barbiturate buffer, at pH 8.5, which separates an

additional small, slower albumin component (A_1) from the A; the sum of A and A_1 may therefore be considered as albumin, the rest as globulin. Under these conditions only one beta component is produced.

Synopsis of cases listed in the table

1. J. C. Duration 2 years. Cardiac failure May–June, 1940 and again January–February, 1942. Contraction of right lung and miliary infiltration of left lung May, 1940 and January, 1942. Pericardial effusion January–February,

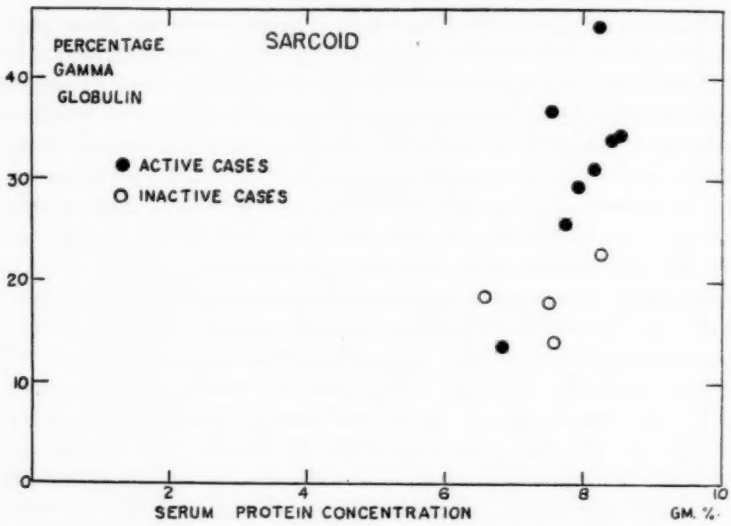

FIG. 1. TOTAL SERUM PROTEIN AND PERCENTAGE OF GAMMA GLOBULIN IN SARCOID
It will be noted that in active cases of the disease there was more regularly a marked increase in gamma globulin than in total proteins

1942. Sarcoid diagnosed by biopsy of inguinal lymph node. Electrocardiogram compatible with myocardial damage. Tuberculin negative. Liver function tests: hippuric acid excreted 0.2 gm. (normal 1.0–1.4 gms.). Galactose tolerance: 0.86 gm. excreted (normal). Analysis done at time of marked clinical activity, with pericardial effusion, myocardial failure, etc.

2. V. M. Duration of disease approximately 2 years. Symptoms of nasal obstruction thought at first to be due to carcinoma. Biopsy by Doctor Proctor showed "hard" tubercles. Sarcoid recognized in these sections and later by lymph

TABLE I

Serum proteins in sarcoid-electrophoretic patterns

PATIENTS	AGE	DATE	TOTAL PROTEINS	TRUE ALBUMIN‡		GLOBULINS‡			SED. RATE§	ACTIVITY OF DISEASE¶	EXTENT OF LESIONS‖
				A	A_1	α	β	γ			
Active											
J. C. (Col.)*	22	1/16/42	7.55	37.2	4.3	12.0	9.8	36.6	31	Very active	Heart (+++); lungs; spleen; skin; liver?
V. M. (Col.)	48	11/24/41	8.84 Alb. 32%†	32.5	3.5	7.2	13.9	42.9		Active	Sinuses; lungs (+++)
		6/ 3/42	8.22 Alb. 39%	30.6	3.3	6.3	14.8	45.1			
D. D. (Col.)	30	12/30/41	8.54 Alb. 44%	39.0	4.5	8.4	13.8	34.3		Active	Spleen (+++); liver; lungs (+++); skin
H. S. (Col.)	25	1/17/42	7.76 Alb. 57%	43.6	4.0	10.1	16.6	25.7		Moderately active	Lungs (+++); glands (++)
C. S. (Col.)	26	1/22/42	7.94 Alb. 51%	41.1	4.8	9.9	15.0	29.3		Active tbc.; active (?) sarcoid	Knee joint (non-tbc.); lungs (sarcoid tbc.) (++); eyes
W. P. (Col.)	25	6/ 5/42	8.43 Alb. 47%	38.6	4.0	6.5	16.8	34.0		Active	(Eyes +++); lungs (++); spleen
F. H. (Col.)	29	3/25/42	8.10 Alb. 38%	39.0	4.2	7.3	17.5	31.9		Moderately active	Lungs (++); spleen; eyes; skin
		5/ 7/42	8.18 Alb. 51%	40.0	3.7	7.2	17.0	31.1	25		
J. G. (Col.)	28	5/20/42	6.82 Alb. 64%	46.1	6.8	14.0	19.5	13.7	21	Moderately active sarcoid; inactive (?) tuberculosis	Lungs (+++); muscles; glands (+++); sinuses (++); bones; eyes

TABLE I—*Concluded*

PATIENTS	AGE	DATE	TOTAL PROTEINS	TRUE ALBUMIN‡		GLOBULINS‡			SED. RATE§	ACTIVITY OF DISEASE¶	EXTENT OF LESIONS‖
				A	A_1	α	β	γ			
Inactive											
M. C. (Wh.)		12/17/41 5/15/42	7.03 6.58 Alb. 66%	43.8 49.7	4.2 4.5	10.7 9.3	16.7 18.3	14.6 18.3	11 (Feb. '41)	Inactive	Mediastinal glands; skin
C. Y. (Wh.)	27	12/27/41	7.60 Alb. 69%	56.3	4.4	11.9	13.4	14.1	7	Inactive	Eyes; lungs (++)
F. T. (Col.)	24	1/16/42	8.28 Alb. 60%	47.6	4.8	11.0	14.0	22.6		Inactive	Skin (+++); lungs spleen; eyes
B. T. (Col.)	15	12/30/41	7.44 Alb. 65%	54.5	3.8	9.3	14.2	18.3		Inactive	Eyes; facial nerves
Normals											
a. Bl.				61.3	3.5	7.0	12.1	16.2			
b. Esk.				55.0	4.0	8.0	16.0	17.0			
c. Biu.				62.4	3.7	9.9	12.4	11.6			

* The patient's color is given in the first column.

† The albumin in percentage as determined by the Howe method is given with the total proteins.

‡ The columns under "True Albumin" and "Globulins" contain the electrophoretic data.

§ The sedimentation rate is given as mm./hr. corrected to normal hematocrit. The normal value does not exceed 10.

¶ The clinical activity of the disease is estimated as accurately as possible, the difficulty of such estimations being obvious.

‖ The last column does not list the complete extent of the disease, only the principal sites or organs involved being indicated.

node biopsy. March, 1941: total proteins 7.63, alb. 45 per cent. Tuberculin negative. Latent syphilis. Disease still active as judged by lung x-ray, skin lesions on legs, nasal symptoms. X-ray of chest unchanged for past year.

3. D. D. Duration 4 years. Splenectomy for "Banti's disease" showed sarcoid in spleen and by liver biopsy August, 1939. Still running fever. Extensive lung involvement by x-ray. Hemoptyses shortly before analysis, with progression of pulmonary lesions. Tuberculin negative. August, 1939 total proteins 8.74, alb. 50 per cent; March 1940 8.08, alb. 46 per cent; December, 1941 8.48, alb. 44 per cent. Bromsulphalein: 10 per cent retention, August, 1939. Definitely an active case clinically.

4. H. S. Duration 4 years. X-ray 4 years ago showed enlarged hilar nodes no symptoms at that time. Few months before routine x-ray taken by draft

board showed extensive infiltration and fibrosis throughout both lungs with enlarged hilar nodes; large soft inguinal and femoral nodes; no symptoms and no fever. Proved by lymph node biopsy. Tuberculin negative. At time of analysis not running fever, but still has marked gland and lung involvement. Considered a moderately active case.

5. C. S. Duration over 3 years. In December, 1938 swelling of knee with fluid in joint; cultures sterile; x-ray showed extensive infiltration in both lungs; old lesions in eyes. Diagnosis proved by lymph node biopsy, 1939. December, 1938 total proteins 6.64, alb. 42 per cent. Tuberculin negative December, 1938; positive 1941. Severe cough and loss of weight October, 1941 and sputum positive for tuberculosis. Cavities then found in chest x-rays in addition to the changes recognized before. At time of blood analysis there had been marked improvement in the tuberculosis but it was probably moderately active. Impossible to estimate the activity of the sarcoid.

6. W. P. Duration 5 years. Almost blind as result of old uveitis. History of rheumatic fever and found to have rheumatic heart disease with mitral stenosis, auricular fibrillation and mild cardiac failure. Dense fibrosis extending from hilum of right lung and some fluid in right pleural cavity. Tuberculin negative. Diagnosis proved by lymph node biopsy. Total proteins March, 1942 8.38, alb. 43 per cent. At time of analysis no change by x-ray and lung signs still present. Clinically an active case.

7. F. H. Duration 5 years. Ocular lesions, probably iritis, at Duke Hospital, 1937. Markedly enlarged spleen removed in South Carolina October, 1940; pathology unknown but probably sarcoid. X-ray here November, 1941 showed extensive pulmonary infiltration and fibrotic lesion as well as enlarged hilar lymph nodes. Diagnosis proved by skin biopsy. Tuberculin negative. Total proteins March, 1942 8.10, alb. 38 per cent. No change and still thought to be in a moderately active phase.

8. J. G. Duration 5 years. First seen 1938 with multiple joint pains, then extensive involvement of muscles with large nodules which later cleared up. Extensive pulmonary involvement by x-ray; marked glandular, skin and sinus involvement. Diagnosed by lymph node biopsy June, 1938. Tuberculin negative 1938. Developed tuberculosis of supraclavicular glands March, 1940 with positive culture and tuberculin then positive. Total proteins March, 1940 7.78, alb. 54 per cent. At time of electrophoresis studies, still extensive lung and lymph node involvement with appearance of some progression of the pulmonary changes and increase in size of the mediastinal nodes. Tuberculosis probably inactive and sarcoid active but impossible to be certain.

9. M. C. Duration about 2 years. Nodule on eye-lid removed in the Wilmer Eye Clinic December, 1940; typical histological picture of sarcoid. Recurrence of this nodule with induration of the tissues about it. Treated with radium and

cleared up in a few months. Physical examination negative except for one other nodule and a palpable spleen. X-ray showed enlarged mediastinal nodes. Total proteins (February, 1941) 6.9, alb. 64 per cent. Blood calcium 11, phosphorus 4. Tuberculin test negative to 0.1 mg., positive to 1 mg. Electrocardiogram normal. Total proteins (December, 1941) 7.03. X-ray (May 5, 1942): mediastinal nodes a little larger. Total proteins (May 14, 1942) 6.58, alb. 66 per cent. Clinically the disease showed little, if any, activity.

10. C. Y. Duration approximately 5 years. At first diagnosed pulmonary tuberculosis. Eyes involved at onset, chronic uveitis. Proved by lymph node biopsy August, 1940. Tuberculin negative. Well now symptomatically and by physical examination. X-ray still shows fibroid infiltration of lungs.

11. F. T. Duration 4 years. Extensive large macular and ulcerative skin lesions in 1938; some fever, palpable spleen. X-ray showed miliary infiltration of the lungs, November, 1938. Process definitely active. Total proteins 9.15, alb. 43 per cent. May, 1941 condition practically cleared up; total proteins 7.80, alb. 61 per cent. Lungs clear by x-ray at time of electrophoresis study; skin lesions healed; spleen no longer palpable. Tuberculin negative. Clinically inactive at time of analysis.

12. B. T. Duration 2 years. Old history of rheumatic fever. Attack of uveitis and facial paralysis 1940. Diagnosis made by lymph node biopsy in 1940. Tuberculin positive 1940. Total protein 1940 8.3, alb. 39 per cent. Perfectly well now with negative chest x-ray and clinically inactive.

RESULTS

From the data of Table I and figure 1 it is seen that the gamma globulin percentage is more consistently elevated in the active phase of sarcoid than is the total protein concentration. In only 4 of the 8 clinically active cases was there a protein concentration greater than 8.0 per cent. However, 7 of these 8 cases showed an elevation of the gamma globulin above the normal value of 14–17 per cent. In some the rise was very marked, reaching 45 per cent in the case of V. M., and exceeding 30 per cent in 5 cases. In all but one of these the elevation of the gamma globulin took place solely at the expense of the albumin, alpha and beta globulins being normal; in the one exception (J. C.) the value of alpha globulin was 12.0 per cent, 10 per cent being considered the maximum normal value. In the one atypical active case (J. G.) the quantity of gamma globulin was normal but there was a moderate elevation of alpha and beta globulins at the expense of albumin.

Of the 4 inactive cases, in contrast, one was found to have normal quantities of gamma globulin and the other 3 showed very little elevation, the highest value being 22.6 per cent. On the other hand, in 3 of the 4 inactive cases there was a slight elevation of the alpha globulin, the values being 10–12 per cent, which was not noted in most of the active cases.

These slight elevations of one or another globulin accounted for the failure of the albumin to reach higher than low normal values in any of the inactive cases. The lowest value for albumin found in the inactive cases (47.6 in F. T.) was nevertheless higher than that of any of the active cases. In only one of the inactive cases was the total protein elevated (8.28 per cent). This was a case in which the protein had been 9.15 per cent when the disease was active 2 years previously, and 7.80 per cent when the condition was inactive one half year before.

It is noted that the relative proportions of the 2 albumins (A and A_1) were normal in all cases except for elevated A_1 in the active case (J. G.) which was atypical in other respects, and in which some other pathological process might well have been present. Comparing the percentage albumin reported by the chemical laboratory by the method of Howe (10) (as shown in Table I under "Total Protein") with the sum of $A+A_1$ obtained by electrophoresis, it is seen that the two determinations parallel each other fairly well, with the chemical determination usually 5–10 per cent too high. This discrepancy is verified by our experience on a large number of normal and abnormal sera. This has also been noted by Luetscher (11) in a variety of abnormal sera and urines, but not in normal sera. The relatively large error of the Howe method is illustrated by the two cases (V. M and F. H.) which were studied at 2 different times. These showed an increase of albumin of 7 per cent and 13 per cent respectively, but practically no change was found in their electrophoretic patterns. In any such discrepancy it may safely be assumed that the electrophoretic determination is the more reliable.

DISCUSSION

The electrophoretic patterns of sera in diseased states have in general been disappointingly non-specific. With the accumulation of knowledge of their variation in clinical and experimental conditions it is expected that eventually there will come understanding of the factors and

sites involved in the production of the various components, but at present they furnish little insight into the nature of a puzzling disease such as sarcoid. Deviations from the normal are caused by malnutrition, loss of protein through the kidney, liver disease, and infections, and probably by unknown factors as well. The complexity of the serum proteins is even greater than is indicated by the multiple electrophoretic components. Actually the five components of different electrophoretic mobilities may be reasonably regarded as chemical genera rather than species of molecules, and hence gamma globulin probably represents a large group of chemically similar but not identical molecules. The slight chemical differences within a group may mediate very important biological effects. As concrete evidence of this we have the fact that the production of immune sera to a variety of antigens may lead to a marked increase in the gamma globulin or sometimes to the appearance of a new peak appearing in a position intermediate between beta and gamma globulin (12) (13). It has been demonstrated by actual separation of this material and by precipitation by the specific antigen that the antibody resides in this elevated globulin fraction (14).

The interpretation of the abnormal patterns here reported is made difficult by the fact that the same component may be elevated in advanced liver disease, presumably in compensation for the impaired formation of albumin. In these cases, however, although the elevation is usually most marked in the gamma fraction, there is a marked tendency for all the globulin fractions to increase. The specificity of the increase in gamma globulin at the expense of albumin in the cases of sarcoid here reported is then presumptive evidence of the response to an infection. This conclusion is consistent with the experimental work with various bacterial antigens and the production of hyperimmune sera, as mentioned previously (12) (13) (14). The added protein may well be antibody although the antigen stimulating its production be undiscovered.

Because of the long disputed relationship between sarcoid and tuberculosis it is of interest to compare the two diseases. Sarcoid appears to be more prone to produce hyperglobulinemia and hyperproteinemia. Of two cases of pulmonary tuberculosis investigated by Luetscher (15) the findings in one were essentially normal and in the

other the gamma globulin was elevated to 32 per cent (plasma) while alpha and beta globulin were normal; however, in this case there was moderate hypoproteinemia (5.7 per cent) in contrast to the normal or high total protein of cases of sarcoid with elevated globulin. Seibert and Nelson (16) report moderate elevation of gamma globulin (20.6 to 28.6 per cent) at the expense of albumin in 3 cases of advanced tuberculosis; the total proteins were normal (6.5 to 7.5 per cent). These cases, then presented a picture similar to our cases of sarcoid, except for the absence of hyperproteinemia. Seibert and Nelson found in experimental tuberculosis in rabbits at first an increase of alpha and beta globulin, and then an increase of gamma globulin which they felt accompanied resistance to the disease.

It is of interest to note that in our cases of sarcoid in which healing had taken place and in which all clinical signs of activity had disappeared, almost normal patterns with only slight decreases of albumin and slight elevation of alpha globulin were found. Elevation of alpha globulin has been reported to occur frequently in acute febrile illnesses (17) (11), but not to our knowledge in patients with sarcoid. It may be noted in passing that experimental sensitization of rabbits to tuberculoprotein has been found to cause an increase in alpha globulin from the rabbit's normal 1–2 per cent to 5–8 per cent (18).

Although we have not followed any case through the process of healing, it is reasonable to suppose that these healed cases also had exhibited the characteristic changes at one time. Study of the blood proteins by the electrophoretic method may prove to be of prognostic value in following the course of the disease.

SUMMARY

1. Electrophoretic patterns for the sera of 12 cases of sarcoid, all of which have been proved by biopsy, are presented.

2. In the 4 cases which showed no clinical signs of activity the sera were almost normal, there being only a slight decrease of albumin and increase of alpha globulin. Those with active lesions were found to have a marked elevation of the gamma globulin at the expense of the albumin, frequently with moderate hyperproteinemia.

3. This electrophoretic pattern is similar to that which has been observed in association with the formation of antibodies in response to an infectious agent.

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ADDITIONAL EVIDENCE OF THE RÔLE OF HYPERSENSITIVITY IN THE ETIOLOGY OF PERIARTERITIS NODOSA

Another Case Associated with a Sulfonamide Reaction

ARNOLD R. RICH

From The Department of Pathology and Bacteriology, The Johns Hopkins Medical School

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The writer has recently reported a series of cases of periarteritis nodosa that occurred in association with hypersensitive reactions following the therapeutic administration of foreign serum and sulfonamides, and has presented evidence that periarteritis nodosa can occur as a manifestation of anaphylactic type hypersensitivity (1). Since the preparation of that paper we have had an opportunity to study another case of periarteritis nodosa following sulfonamide therapy. Because of the importance of the matter for the understanding of the pathogenesis of periarteritis nodosa, and also because of the widespread use of sulfonamides, and because the present case affords particularly persuasive evidence that the periarteritis nodosa was not present before the hypersensitive reaction to the sulfonamide occurred, it is regarded as desirable to place this additional case on record.

The patient (Johns Hopkins Hospital History No. 246274) was a negro male, 66 years of age. He came to the dispensary complaining of an ulcer on the scrotum. He stated that during the first World War he "had gotten steel wool and acid" on his scrotum, and that since then the skin of the scrotum had been "patchy white and rough." Four months before coming to the dispensary he was bitten on the scrotum by a tick. He pulled the tick off, the site became infected and an ulcer developed, which persisted. Examination of the scrotum disclosed patchy leucoplakia and a punched out ulcer with indurated edges. A biopsy was made, which showed squamous cell carcinoma. The patient did not return to the dispensary until five months later. The carcinomatous ulcer had increased in size. He was admitted to the hospital and a radical operation for removal of the carcinoma and the inguinal lymph nodes was performed. The patient was given 1 gm. of sulfathiazole the day before the operation as a prophylactic measure, since the ulcerated area was infected. On the day of the operation he received 2 gms. of the drug; 4 gms. were administered on each of the two following days, and 3 gms. on the fourth post-operative day. The drug was then discontinued. On the eighth post-operative day the patient complained of stiffness and pain in the neck

and pain in the eyes. On the eleventh post-operative day 4 gms. of sulfathiazole were placed in the operative wound, and sulfathiazole by mouth was started again. 2 gms. were given on this day. Bilateral conjunctivitis developed, and in the evening of this day the temperature, which had never reached 102°F. previously and which, for three days, had not reached 101°F., rose to 102.3°F., and each day thereafter it attained a level between 102 and 103°F., until the last two days of life, when the drug was discontinued. On the twelfth day the patient was described as being very sick. An ophthalmological consultant regarded the conjunctivitis as probably a sulfonamide reaction. The patient continued to receive sulfathiazole, 5 gms. on the twelfth post-operative day, 3 gms. on the thirteenth, 4 gms. by mouth and 1 gm. intravenously on the fourteenth; 6 gms. on the fifteenth, and 3 gms. on the sixteenth day. On the thirteenth day the patient was in a semi-conscious condition. A blood culture showed less than 1 colony of staphylococci per cc. at the end of 48 hours incubation, and on the fourteenth day one colony per cc. (staphylococci were not found in the autopsy blood cultures). On the fifteenth day the blood non-protein nitrogen was 44 mg. per cent. No subsequent determinations were made. A number of blood transfusions were given during the post-operative course. Death occurred on the eighteenth post-operative day.

At autopsy (no. 17895), performed by Dr. G. G. Merrill, the operative site was found to be infected. There was haemorrhage in the intestinal lumen, the site of origin of which was not discovered. There were multiple intestinal polyps, but none of these were ulcerated or haemorrhagic. The prostate was moderately enlarged by benign hypertrophy, and on microscopic examination it was found to contain a small adenocarcinoma. (An area of induration suspicious of carcinoma was noted on rectal examination at his first dispensary admission.) The testes were atrophic. The lungs were oedematous. No other significant lesions were noted macroscopically.

Microscopically, there were widespread, fresh lesions of *periarteritis nodosa*. These vascular lesions were found in the tissues at the operative site, in the heart, liver, spleen, kidneys, testis, bladder and prostate. None were found in the sections of the gastro-intestinal tract. The vascular lesions were, in every respect, perfectly characteristic of periarteritis nodosa. There was necrosis, fibrinoid alteration and hyalinization of the media, with a marked perivascular infiltration of mononuclear and polymorphonuclear cells, including many eosinophils. Some of the affected vessels were thrombosed. The lesions were precisely like those described and abundantly illustrated in our previous paper on this subject (1). They were extremely profuse in the kidneys and in the bladder.

In addition to these extensive vascular lesions, there was a *diffuse, inflammatory infiltration of the myocardium*, composed of mononuclear and polymorphonuclear cells, including eosinophils, quite like that described by French and Weller (2) in patients treated with sulfonamides. This was observed also by the present writer in several of the cases of periarteritis nodosa developing in patients who had hypersensitive reactions following foreign serum and sulfonamide therapy (1). In the present case it is interesting that there was also an extensive, diffuse, interstitial

infiltration of precisely the same character, rich in eosinophils, in the *kidneys*; and the *pancreas* was involved in this manner to an extraordinary degree.

Finally, there were present in the lungs, spleen, kidneys and bladder very minute *focal necroses densely infiltrated and surrounded by mononuclear and polymorphonuclear cells, including eosinophils*, quite like those encountered by Lederer and Rosenblatt (3), Merkel and Crawford (4) and the present writer (1) in patients dying following sulfathiazole therapy. The necroses in these minute focal areas appear to develop as a result of damage to minute arterioles and capillaries. In the present case the abundance of lesions in vessels of this calibre provided an exceptional opportunity to observe that these little focal inflammatory infiltrations develop immediately about a necrotic, minute vessel. In the lung, in which the inflammatory cells can escape into the alveoli and therefore do not crowd so densely over the capillaries, necrosis of the capillary walls, and thrombosis, is evident in the centers of the inflammatory foci. In the present case there was marked damage of numerous glomerular capillaries.

The case described above represents the eighth instance of periarteritis nodosa that we have observed in patients dying following treatment with foreign serum, foreign serum and sulfonamide therapy, or sulfonamide therapy alone. Evidence that this type of vascular lesion is a result of hypersensitivity was presented in some detail in our previous paper (1). In the present case there was a rise in temperature on the ninth day following the initial dose of sulfathiazole, when treatment with the drug was resumed following a five day lapse. The relation of hypersensitivity to the sulfonamide febrile reaction has been pointed out by Lyons and Balberor (5). The patient also developed conjunctivitis, which is a familiar manifestation of hypersensitivity to the sulfonamides. No skin rash was noted, but the patient was a negro.

In the series of cases previously reported by the writer (1), there was very strong evidence that the development of the periarteritis nodosa was associated with the hypersensitive reactions to the foreign serum or sulfonamides that the patients received, and was not merely a coincidence. The present case is of particular importance in this regard for, aside from the freshness of the vascular lesions which stamped them as being of unmistakably recent origin, there was an opportunity to examine (a) tissue taken at biopsy from the scrotum five months before the sulfonamide was administered; (b) tissue taken from the same scrotal region, and from the inguinal nodes, at operation one day after the first dose of sulfonamide was administered and one week before the

sulfonamide reaction (rise in temperature; conjunctivitis) occurred; and, finally, (c) tissue at the site of operation, and elsewhere, at autopsy seventeen days following the operation and nine days following the hypersensitive reaction. It is significant that there were no lesions of periarteritis nodosa in the biopsy of the scrotum, none in the scrotal tissue or in the inguinal nodes removed at operation, but numerous vascular lesions of this type in the operative wound and in the immediately adjacent scrotal tissues at the time of autopsy. It is doubtful whether it will be possible to obtain in any patient any better evidence that the vascular lesions found in cases of this type develop in association with the hypersensitive reaction, and are not unrelated lesions that were present coincidently before the serum or drug therapy was instituted.

SUMMARY

In a previous paper the writer described a series of cases in which periarteritis nodosa was found at autopsy in patients who had had hypersensitive reactions resulting from foreign serum and sulfonamide therapy, and evidence was presented that periarteritis nodosa can be a manifestation of the anaphylactic type of hypersensitivity. The present report describes an additional case, in which periarteritis nodosa developed following a reaction (fever; conjunctivitis) to sulfathiazole.

This case is of particular interest for the reason that there was an opportunity to examine tissue removed from the patient's scrotum at five months and at one week before the sulfonamide reaction occurred, and at nine days following the reaction. Periarteritis nodosa was not present in the specimens of scrotum removed before the reaction occurred. It was present there, and widespread throughout the body, following the reaction.

This case, in conjunction with the series described in the previous paper, is a further indication, derived from clinical and pathological material, that periarteritis nodosa is a manifestation of anaphylactic type hypersensitivity. In experiments carried out with Dr. John E. Gregory, to be reported presently, typical visceral periarteritis nodosa has been produced by the intravenous injection of a single, large amount of foreign serum into the normal animal, thus providing the

opportunity for a protracted circulation of antigen while hypersensitivity develops.

These observations indicate that the continued administration of a sulfonamide or of foreign serum after symptoms of hypersensitivity have appeared, or the injection of a single large amount of foreign serum, carries the danger of producing vascular damage of the periarteritis nodosa type.

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BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal.)

Essentials of Pathology. By LAWRENCE W. SMITH and EDWIN S. GAULT. 2nd ed. 960 pp. Illus. $10.00. *D. Appleton-Century Company, New York and London*, 1942.

This text-book of pathology is divided into 1) general pathology, 2) tumors, and 3) systemic pathology. This necessitates much useless repetition. As a reference book it is frequently difficult to find the main account of a subject which in some instances is under general pathology and at other times under diseases of a specific system. The subjects of rheumatic fever, tuberculosis and syphilis suffer especially by subdivision into too many chapters. Congenital syphilis is particularly incomplete.

The addition of case histories helps to integrate the widespread effects of a single etiologic agent. This method would be more successful in correlating clinical and pathological features, however, if cases with complete rather than partial autopsies were utilized. For example, the association of pericarditis with nephritis is nowhere noted.

The bibliography while not complete is adequate and includes recent contributions. Unfortunately, it is arranged alphabetically by chapters instead of by subjects, making it difficult to ferret out pertinent references.

The subject material is as complete as in any recent text-book of pathology. However discussions of unknown etiologic agents are far too brief, as are reports of experimental work throwing light on obscure questions. Other omissions include especially immunity and allergy; Fiedler's myocarditis, sarcoid and lupus erythematosus disseminata. Diseases of the skin have been omitted entirely.

Especially to be praised are the many excellent illustrations and color prints. These remarkably complete histopathological illustrations make this book particularly valuable as an aid to the laboratory study of microscopical pathological preparations.

E. H. O.

Debatable Tumours in Human and Animal Pathology. By W. F. HARVEY, E. K. DAWSON, and J. R. M. INNES. 124 pp. 40 plates. 10 sh. 6d. *Oliver and Boyd, Edinburgh and London*, 1940.

This volume is just another treatise on the morphology of tumors. It deals with 9 "debatable" neoplasms such as seminomas, meningiomas, melanomas, etc. There is little, however, that cannot be found in any standard text-book on the subject.

R. H. F., JR.

Necropsy. By BÉLA HALPERT. 75 pp. $1.50. *The C. V. Mosby Company, St. Louis*, 1941.

This guide should prove useful to the second year medical student. The autopsy technique is, with a few minor exceptions, similar to that employed at the Johns Hopkins Hospital. Appended are two typical protocols with microscopic notes.

R. H. F., JR.

Synopsis of the Preparation and Aftercare of Surgical Patients. By HUGH C. ILGENFRITZ, RAWLEY M. PENICK, JR., and URBAN MAES. 532 pp. Illus. $5.00. The C. V. Mosby Company, St. Louis, 1941.

This excellent pocket-sized manual of pre- and postoperative care adequately covers a field in which general surgery has made notable progress in recent years. The authors, like good householders, have brought forth from their treasures things both new and old. Commendable discretion has been used in the selection of material, only those therapeutic measures being included which are of demonstrable value. Fads and personal enthusiasms have been successfully avoided. The more common diseases and complications have been considered from the standpoint of treatment, and therapy has been presented as a rational scientific procedure based upon accurate knowledge of the normal and pathologic physiology of the patient. The format is excellent; the text clear and concise. A valuable feature of the book is the well chosen list of references at the end of each chapter which enables the reader to find additional information on particular subjects. The interne will find this handbook a useful guide and experienced surgeons will read it with interest and profit.

P. B. P.

The Reception of William Beaumont's Discovery in Europe. By GEORGE ROSEN. 97 pp. $5.00. *Schuman's, New York*, 1942.

Any publication relating to the revolutionary work of William Beaumont will inevitably arrest the attention of every student of medicine; and Dr. Rosen's alluring title is therefore certain to attract the medical profession as a whole.

A fascinating account of the manner in which Alexius St. Martin received his wound and the events leading up to the immediate treatment and later study by Beaumont constitutes a most interesting part of the publication. In a detailed presentation the author shows the ardent, scientific interest which Beaumont exercised in his persistent efforts to keep in constant contact with St. Martin, though in this he was unfortunately only partially successful.

The author presents convincing evidence of the influence which the rather crude but brilliant work of this obscure military surgeon exerted upon the medical profession in Europe. We are told that as early as 1826 articles dealing with Beaumont's work were published in Germany, and that not many months thereafter they appeared in the Edinburgh Journal of Medical Science; about two years

later the work was discussed in a French journal, and soon after in an Italian publication. A German edition of Beaumont's book was published at Leipzig in 1834, and was favorably reviewed by various critics. Schmidt concluded his review by stating, "This is enough, not to satisfy the reader, but to stimulate him to study in detail a book which contains on every page materials worth examining, and which is generally important for physiology, pathology, dietetics, and therapy."

Johannes Muller, Schultz, Schwann and other prominent European observers were quite lavish in their praise of the overwhelming importance of Beaumont's researches. In addition to German recognition, English medical literature accorded most favorable attention. The author cites the great interest shown by the celebrated French physiologist, Claude Bernard, and the great importance he attached to Beaumont's findings.

The reviewer entirely agrees with the author when he states in his conclusion that William Beaumont was undoubtedly one of the most interesting personalities in nineteenth century medical history, and that his researches provided a powerful impulse for the further development of gastric physiology, pathology and therapy. The author is to be congratulated on his splendid, convincing, and valuable contribution to medical commentary.

E. H. G.

Carcinoma and Other Malignant Lesions of the Stomach. By WALTMAN WALTERS, HOWARD K. GRAY, and JAMES T. PRIESTLEY. 576 pp. Illus. $8.50. *W. B. Saunders Company, Philadelphia and London*, 1942.

Walters, Gray, Priestly and their confreres present a monograph of inestimable value to the medical profession.

Its first brief but illuminating chapter deals with the history of carcinoma of the stomach, ably presented by Walter C. Alvarez; the last chapter develops prognosis and results, and the book abounds in the authentic information so greatly needed by the medical profession in its war on this dread disease.

Morlock and Hartman present the crux of the cancer problem when they state that, if results in the treatment of malignant gastric lesions are to show improvement, more responsibility must be assumed by the clinician first consulted by the patient. McCarty makes timely mention of the important fact that early carcinoma of the stomach gives no textbook picture of signs or symptoms, that it begins as a small, curable lesion which it is the clinician's responsibility to recognize. An appalling large percentage of cancer cases are treated medically for many months before being referred to a surgeon. On the other hand, there are many successes when early diagnosis is made and surgery promptly applied.

The presentation of digestive symptoms is most illuminating, and the importance of intense and continued study of every case of "indigestion" is stressed, in the realization that each and every one may be a harbinger of malignant disease.

In the chapters on gastroscopy, laboratory observations, indications for treat-

ment, special diagnostic problems and observations, as well as others of importance in this field, one will find a veritable gold mine of helpful information.

This reviewer considers the monograph the most brilliant presentation of this subject yet given. It is indeed a classic, and should therefore be in the hands of every student and member of the medical profession.

E. H. G.

Report of Medical Advisory Committee on the Use of Mass Miniature Radiography in the Detection of Pulmonary Tuberculosis among Recruits for H. M. Forces. Cmd. 6353. 6 pp. Five cents. His Majesty's Stationery Office, London, 1942.

From this small pamphlet we learn that miniature radiography is being carried out on a rather large scale in the British Navy and to a less extent in the Air Force and Army. The results indicate that this is the most suitable method of applying radiological examinations where there are large numbers of troops or recruits to be examined. The Committee looks forward to the extension of this service over a large field.

A. M. F.

INDEX TO VOLUME LXXI

Pagination According to Months:

July, 1942, pp. 1–46
August, 1942, pp. 47–122
September, 1942, pp. 123–190
October, 1942, pp. 191–252
November, 1942, pp. 253–314
December, 1942, pp. 315–388

Book Reviews 45, 118, 184, 247, 307, 380

Chambers, John W. (see Ravitch, Mark M.) 299
Cochlea to the cerebral cortex of the cat, Topical projection of nerve fibers from local regions of the 315

Davis, B. D. (see Fisher, A. M.) 364
Davis, John S. and Edward S. Stafford: Successful construction of an extrathoracic esophagus 191

Endometrial hyperplasia, An evaluation of progesterone therapy in the treatment of .. 282
Esophagus, Successful construction of an extrathoracic 191

Finney, Dr. J. M. T., Sr., Minute adopted by the Medical Board, June 29, 1942 47
Finney, General John M. T., Message from the 18th General Hospital concerning the death of 246
Fisher, A. M. and B. D. Davis: The serum proteins in sarcoid. Electrophoretic studies 364
Fixative for general use, A dehydrating, including a description of techniques and stains for paraffin and celloidin sections 96
Fleischmann, Walter and Harris B. Schumacker, Jr.: The relationship between serum cholesterol and total body cholesterol in experimental hyper- and hypothyroidism 175
Follis, R. H., Jr.: Myocardial necroses in rats on a potassium low diet prevented by thiamine deficiency 235
Follis, R. H., Jr. (see Rich, Arnold R.) 345
Follis, R. H., Jr. (see Wintrobe, Maxwell M.) 141

Ham, George C. and Morris Rosenfeld: Ultracentrifugation of the antidiuretic, chloruretic and pressor factors of posterior pituitary extracts 18
Harvey, A. M. and J. L. Lilenthal, Jr.: Observations on the nature of tetany. The effect of adrenaline 163
Holt, L. Emmett, Jr. (see Josephs, Hugh W.) 84
Humphreys, Stewart (see Wintrobe, Maxwell, M.) 141
Hyper- and hypothyroidism, The relationship between serum cholesterol and total body cholesterol in experimental 175

Jones, G. E. Seegar and R. W. Te Linde: An evaluation of progesterone therapy in the treatment of endometrial hyperplasia..... 282
Josephs, Hugh W.: Factors influencing the level of vitamin A in the blood of rats.. 253
Josephs, Hugh W.: Studies in vitamin A. Influence of vitamin A on serum lipids of normal and deficient rats..... 265
Josephs, Hugh W., L. Emmett Holt, Jr., Herbert C. Tidwell and Charlotte Kajdi: The influence of dietary fat upon the excretion of urobilin..... 84

Kaiser, Irwin H.: The specificity of periarterial fibrosis of the spleen in disseminated lupus erythematosus..... 31
Kajdi, Charlotte (see Josephs, Hugh W.)..... 84

Levy, Dexter S. (see Scherlis, Sidney)..... 24
Lilienthal, J. L., Jr. (see Harvey, A. M.)..... 163
Longmire, William P. (see Price, Philip B.)..... 51
Lupus erythematosus, disseminated, The specificity of periarterial fibrosis of the spleen in..... 31

Miller, Mitchell H. (see Wintrobe, Maxwell M.)..... 141
Myocardial necroses in rats on a potassium low diet prevented by thiamine deficiency..... 235

Najjar, Victor (see Wintrobe, Maxwell M.)..... 141

Periarteritis nodosa, The rôle of hypersensitivity in..... 123
Periarteritis nodosa, Additional evidence of the rôle of hypersensitivity in the etiology of..... 375
Pickrell, Kenneth L.: A sulfonamide film for use as a surgical dressing. Preliminary report..... 304
Pituitary extracts, posterior, Ultracentrifugation of the antidiuretic, chloruretic, and pressor factors of..... 18
Plasma volume determinations, The use of T-1824 in..... 51
Price, Philip B. and William P. Longmire: The use of T-1824 in plasma volume determinations..... 51

Ravitch, Mark M. and John W. Chambers: Spirochaetal survival in frozen plasma.. 299
Rich, Arnold R.: The rôle of hypersensitivity in periarteritis nodosa..... 123
Rich, Arnold R.: Additional evidence of the rôle of hypersensitivity in the etiology of periarteritis nodosa..... 375
Rich, Arnold R. and R. H. Follis, Jr.: The effect of low oxygen tension upon the development of experimental tuberculosis..... 345
Rosenfeld, Morris (see Ham, George C.)..... 18

Sarcoid, The serum proteins in. Electrophoretic studies..... 364
Scott, Virgil: Note on absence of syphilitic reagin and antigenic substance in urine.. 242
Scherlis, Sidney and Dexter S. Levy: Investigation into the mechanism of the Weltmann serum coagulation reaction. A preliminary report..... 24
Shumacker, Harris B., Jr. (see Fleischmann, Walter)..... 175

Shumacker, Harris B., Jr.: Sympathetic denervation of the feet and legs occurring spontaneously or as a result of disease. A preliminary report 1
Spirochaetal survival in frozen plasma 299
Stafford, Edward S. (see Davis John S.) 191
Stein, Harold J. (see Wintrobe, Maxwell M.) 141
Sulfonamides, Comparative activity of, against coliform bacteria in the intestines of mice 213
Sulfonamide film for use as a surgical dressing. Preliminary report 304
Sympathetic denervation of the feet and legs occurring spontaneously or as a result of disease. A preliminary report 1
Syphilitic reagin and antigenic substance, Note on absence of, in urine 242

Te Linde, R. W. (see Jones, G. E. Seegar) 282
Tetany, Observations on the nature of. The effect of adrenaline 163
Thiamine deficiency in swine, A study of. Together with a comparison of methods of assay 141
Tidwell, Herbert C. (see Josephs, Hugh W.) 84
Tuberculosis, The effect of low oxygen tension upon the development of experimental 345

Urobilin, The influence of dietary fat upon the excretion of 84
U. S. Army General Hospitals 18 and 118 organized at the Johns Hopkins Hospital .. 112

Vandegrift, William B.: A dehydrating fixative for general use, including a description of techniques and stains for paraffin and celloidin sections 96
Vitamin A, Factors influencing the level of, in the blood of rats 253
Vitamin A, Studies in. Influence of vitamin A on serum lipids of normal and deficient rats 265

Walzl, E. M. (see Woolsey, C. N.) 315
Weltmann serum coagulation reaction, Investigation into the mechanism of 24
White, Harold J.: Comparative activity of sulfonamides against coliform bacteria in the intestines of mice 213
Wintrobe, Maxwell M., Harold J. Stein, Mitchell H. Miller, Richard H. Follis, Jr., Victor Najjar and Stewart Humphreys: A study of thiamine deficiency in swine. Together with a comparison of methods of assay 141
Woolsey, C. N. and E. M. Walzl: Topical projection of nerve fibers from local regions of the cochlea to the cerebral cortex of the cat 315

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Topical Projection of Nerve Fibers from Local Regions of the Cochlea to the Cerebral Cortex of the Cat. CLINTON N. WOOLSEY AND EDWARD M. WALZL 315

The Effect of Low Oxygen Tension upon the Development of Experimental Tuberculosis. ARNOLD R. RICH AND RICHARD H. FOLLIS, JR. 345

The Serum Proteins in Sarcoid: Electrophoretic Studies. A. MURRAY FISHER AND BERNARD D. DAVIS 364

Additional Evidence of the Rôle of Hypersensitivity in the Etiology of Periarteritis Nodosa. Another Case Associated With a Sulfonamide Reaction. ARNOLD R. RICH 375

Book Reviews 380

Index to Volume LXXI 385

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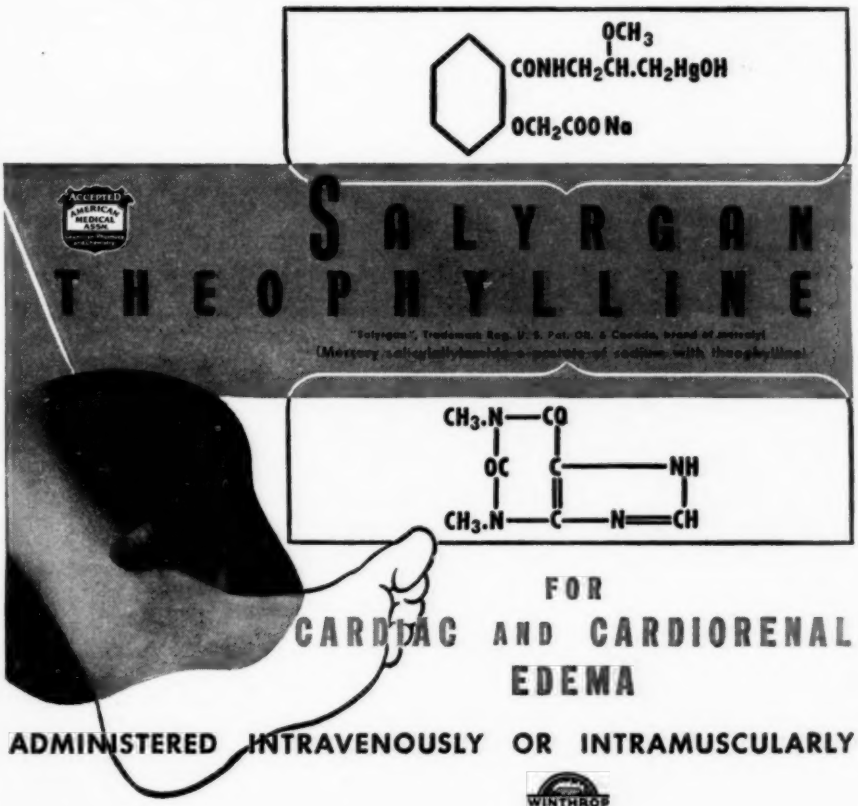

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